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FSUM 10551.1  
PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Robert A. Holton et al.

ART UNIT 1625

Serial No. 09/978,436

Filed October 16, 2001

Confirmation No. 5339

For RADIOSENSITIZING TAXANES AND THEIR PHARMACEUTICAL PREPARATIONS

**DECLARATION OF PRIOR INVENTION UNDER 37 C.F.R. ' 1.131**

We, Robert A. Holton, Hossain Nadizadeh and Li-Xi Yang declare as follows:

1. We are the inventors of the subject matter claimed in the above-entitled United States patent application.
2. We are submitting this Declaration to establish completion of our invention in the U.S. before June 7, 1995, the filing date of U.S. Patent No. 5,780,653.
3. We reduced to practice the invention claimed in this application in the United States before June 7, 1995.
4. Facts in support of this Declaration are attached hereto as Exhibit A. Exhibit A is a true and correct copy of experimental note book page numbers 1-6, 8-14, 16, 18, 20, 22-28, 30-38, 40, 42-44, 46, 48, 50, 52, 54-62, 64, 66, 68-74, 76-78, pages 1-4 of "Taxol and TM 24 hrs prior Xray," pages 1-3 of "TM drug,"

FSUM 10551.1  
PATENT

pages 1-3 of "TNB," "TNB+22Gy" and "TNB control" (with dates deleted), upon which Figures 1-18 and Examples 4.1, 4.2, 4.4, 4.5, 4.6 of this application are based. Specifically, compound taxol-metro (or TM) corresponds to N-debenzoyl-N-(t-butylcarbamoyl)-7-(metronidazoleoxycarbonyl)taxol of Example 2 and nitrophenyl taxol (or taxol nitrobenzene or TNB) corresponds to N-debenzoyl-N-(t-butylcarbamoyl)-3'-desphenyl-3'-(4-nitrophenyl)taxol of Example 3 of this application. As such, Exhibit A evidences our reduction to practice of the invention claimed in the application prior to June 7, 1995.

5. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. ' 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

  
Robert A. Holton

4/28/03  
Date

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Hossain Nadizadeh

\_\_\_\_\_  
Date

\_\_\_\_\_  
Li-Xi Yang

\_\_\_\_\_  
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Robert A. Holton

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Date

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Hossain Nadizadeh

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Date  

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Li-Xi Yang

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4/28/03  
Date

FSUM 10551.1  
PATENT

18 and Examples 4.1, 4.2, 4.4, 4.5, 4.6 of this application are based. Specifically, compound taxol-metro (or TM) corresponds to N-debenzoyl-N-(t-butylcarbamoyl)-7-(metronidazoleoxycarbonyl)taxol of Example 2 and nitrophenyl taxol (or taxol nitrobenzene or TNB) corresponds to N-debenzoyl-N-(t-butylcarbamoyl)-3'-desphenyl-3'-(4-nitrophenyl)taxol of Example 3 of this application. As such, Exhibit A evidences our reduction to practice of the invention claimed in the application prior to June 7, 1995.

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Robert A. Holton

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Date

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*Nadizadeh*

Hossain Nadizadeh

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4/28/03

Date

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Li-Xi Yang

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Date

metronidazole - CL and metronidazole - I Toxicity under O<sub>2</sub>/N<sub>2</sub>

$358/8 = 4.4 \times 10^4$ ,  $1+2+1 = 2 \times 10^3$ ,  $0.1+2.1 = 2 \times 10^9$ ,  $1+9 = 2000$

$4.5 + 40.5 = 200$  Each dish: 15  $\mu$ l drug solution + 2 ml HBSS

200 cells/dish incubated 1h per 1h

Drugs: solvent: DMSO. 15  $\mu$ l drug solution later added into 2 ml HBSS

metro-CL stock: 1: 1.06 M, 2: 0.638 M, 3: 0.1276 M

metro-I stock: 1: 0.476 M, 2: 0.0952 M, 3: 0.01904 M

Groups	Final Conc (mM)	N <sub>2</sub> Colonies	O <sub>2</sub> Colonies	mean N <sub>2</sub> /35	SF 0.68	SF/PE
0	0	135	126, 160, 151	140.7	0.73	
metro-I				N <sub>2</sub> : 7.3, 0.037		0.054
1	3.54	15, 4, 3	124, 115, 125	121	0.61	0.831
				N <sub>2</sub> : 22, 0.11		0.162
2	0.71	22	150, 129, 153	144	0.72	0.986
				N <sub>2</sub> : 39.7, 0.198		0.292
3	0.14	28, 45, 46	87, 117, 138	114	0.57	0.781
				N <sub>2</sub> : 11.4, 0.57		0.781
metro-CL				N <sub>2</sub> : 38, 0.19		0.279
1	7.89	41, 35	116, 103, 110	109.6	0.548	0.75
				N <sub>2</sub> : 99, 0.495		0.728
2	4.75	99	152, 151, 159	154	0.77	1.05
				N <sub>2</sub> : 136.7, 0.683		1.06
3	0.95	130, 156, 124	156, 151, 147	154.3	0.767	1.04

Cytotoxicity for metro-Cl, metro-I, metro-CHO under Nr/02.

Drugs: metro-Cl and metro-I were prepared on '74.

metro-CHO: 2.655M stock.

dilutions: 1. 19.5ul <sup>stock</sup> + 26ml HBSS = 19.76mM

2. 97.5ul + 26ml HBSS = 9.919mM

Each dish Plated 200 cells. 3. 48.75ul + 26ml HBSS = 4.97mM.

N<sub>2</sub> Colonies in Dish

O<sub>2</sub> Colonies in Dish

M-CHO 1. 0

0

M-CHO 2. 0

0

M-CHO 3. 0

0

N<sub>2</sub> Count

mean

SF

SF/PE

O<sub>2</sub> Count

mean

SF

SF/PE

-Cl 3. 122. 109. 119.

118.7

0.59

0.91

156. 136. 159.

150.3

0.75

1.00

M-cl 2. 122. 112. 121.

118.3

0.59

0.91

142. 140. 130

137.3

0.69

0.92

M-C 3. 100. 86. 90

92

0.46

0.71

107. 125. 117

116.3

0.58

0.78

M-I 3. 133. 145. 134

137.3

0.69

1.06

136. 132. 140

136.

0.68

0.91

M-I 2. 101. 103. 101

101.7

0.51

0.78

146. 156. 128

143.3

0.72

0.96

M-I 1. 45. 101. 103

49.7

0.50

0.77

156. 136. 159

150.3

0.75

1.00

(PE)

(PE)

0. 131. 127. 130

129.3

0.65

1.59

152. 139

150

0.75

Cytotoxicity of (actin)<sub>2</sub>N(CH<sub>2</sub>)<sub>3</sub>N(actin)<sub>2</sub>

Drug:  $0.3772/0.3 \text{ ml}$ ,  $d = 1.2472/\text{ml}$ ,  $= 1.83 \text{ M}$ . stock.

Working dilutions: 1.  $13.5 \text{ ml} + 1.9 \text{ ml HBSS} = 12.91 \text{ mM}$ .

2.  $67.5 \text{ ml} + 1.9 \text{ ml HBSS} = 6.48 \text{ mM}$

3.  $33.75 \text{ ml} + 1.9 \text{ ml HBSS} = 3.24 \text{ mM}$

Results:	N <sub>2</sub>	O <sub>2</sub>
Colony	0. 129, 109, 126.	135, 112, 110
1.	0 0 0	0 0 0
2.	0	0
3.	2, 5, 5,	0

# 1. Radiosensitization of metronidazole-CL at 7.82 mM

Radiation dose (minutes)	Hypoxia + Drug cells	Hypoxia cells	Oxic + Drug cells	Oxic cells
20 Gy (10')	20,000	10,000		
12 Gy (6')	2,000	1,000		
6 Gy (3')	1,000	500		
0	200	200		
8 Gy (4')			20,000	20,000
6 Gy (3')			5,000	5,000
4 Gy (2')			1,000	1,000
0			200	200

## 2. Cytotoxicity of metronidazole-aldehyde under O<sub>2</sub> conditions

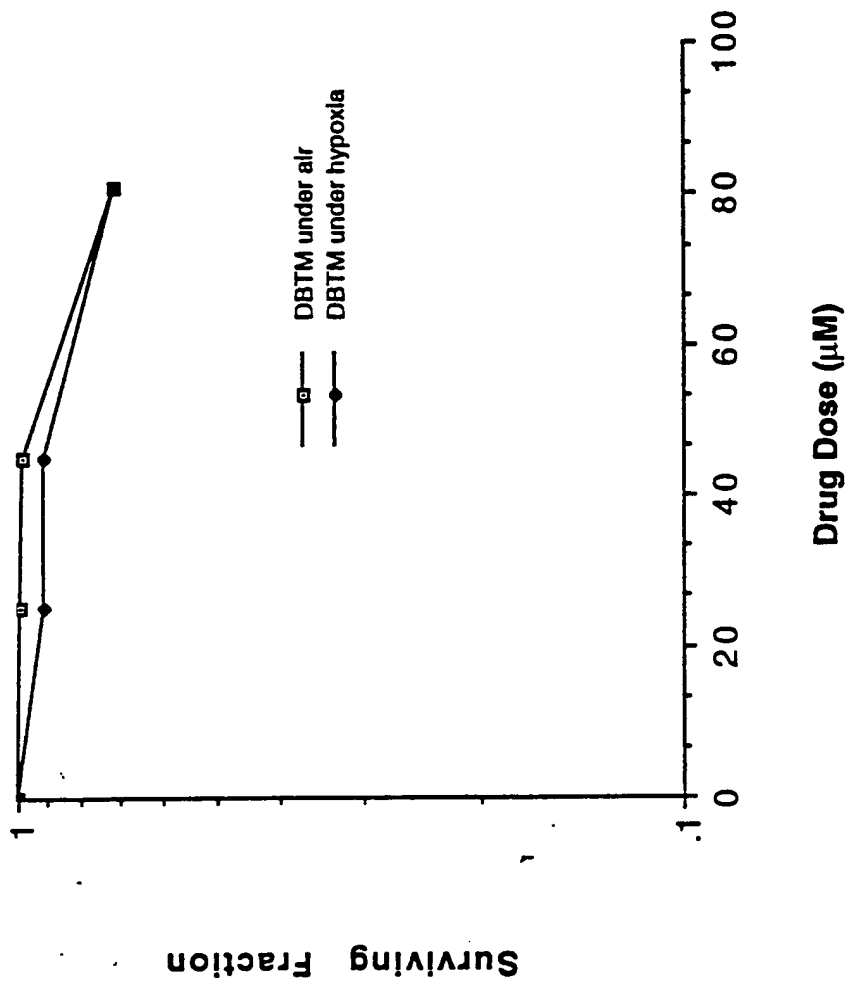
Each dish contains 200 cells, each group 3 dishes

Final Conc: #1: 0.01 mM. #2: 0.1 mM. #3: 1 mM. Treated for 2h.

Count #	N <sub>2</sub> + Drug	mean	SF	SF/PE	N <sub>2</sub>	mean	SF	SF/PE
20 Gy	2. 2. 2	2	0.0001	0.00027	57.69.58	61.3	0.006	0.136
12 Gy	13. 4. 8	10	0.005	0.0135	63.56.57	58.7	0.0587	0.139
6 Gy	103. 46. 57	87	0.087	0.235	121. 90. 50	87	0.174	0.39
0	48. 83. 92	74.3	0.37	(PE)	104. 117. 50	90.3	0.45	(PE)
O <sub>2</sub> + Drug		mean	SF	SF/PE	O <sub>2</sub>	mean	SF	SF/PE
20 Gy	240. 203. 246	229.7	0.01	0.027	280. 291. 290	287	0.014	0.026
6 Gy	176. 159. 173	169.3	0.0339	0.095	273. 280. 260	271	0.0542	0.099
0	75. 76. 70	73.7	0.37	(PE)	116. 106. 114	110.3	0.55	(PE)

# Toxicity of DBTM on CHO Cells

(ac 4R)



Cytotoxicity for (nitro)<sub>2</sub>N(CH<sub>2</sub>)<sub>3</sub>-4 N (nitro), abbreviated to 4C and 3C under 1/2 O<sub>2</sub> conditions. After drug was added into dishes, cells were made hypoxic for 1h at room temperature, then incubated at 37°C under hypoxia for another 1h, totaling 2h foroxic cells, treated with drug for 2h in incubator, irradiated at 19', 25%/min.

Group	drug	N <sub>2</sub> /O <sub>2</sub>	Radiation Dose (Gy)	Drug Conc. (mM)
1	4C	N <sub>2</sub>	20	0.81
2	4C	N <sub>2</sub>	12	0.045
3	4C	N <sub>2</sub>	6	0.0025
4	4C	O <sub>2</sub>	8	0.81
5	4C	O <sub>2</sub>	6	0.045
6	4C	O <sub>2</sub>	4	0.0025
7	3C	N <sub>2</sub>	20	1 mM
8	3C	N <sub>2</sub>	12	0.1
9	3C	N <sub>2</sub>	6	0.01
10	3C	O <sub>2</sub>	8	1-
11	3C	O <sub>2</sub>	6	0.1
12	3C	O <sub>2</sub>	4	0.01

Control #	N <sub>2</sub>	mean	SF	SF/PE	O <sub>2</sub>	mean	SF	SF/PE
4C (1)	88.91.116	48.3	0.44	0.71	98.106.86	96.7	0.48	0.71
4C (2)	123.120.135	126	0.63	0.91	138.127.136	133.7	0.67	0.98
4C (3)	63.132.142	112.3	0.56	0.81	132.138.134	134.7	0.67	0.99
3C (1)	46.45.131	107.3	0.54	0.78	23.35.35	31	0.155	0.23
3C (2)	56.56.71	61	0.305	0.44	106.96.135	112.3	0.56	0.83
3C (3)	36.61.37	44.7	0.22	0.32	94.123.94	103.7	0.52	0.76
0	136.140.139	138.3	0.67	(PE)	142.126.140	136	0.68	(PE)

# 1. Radiosensitization of CHO cells by metronidazole-I. (M-I)

Drug M-I : from stock 1.12M, get 0.5 ml, dilute with 0.75 ml DMSO to make a M-I solution of 0.476M, from which 0.54 ml was taken, added into 71.56 ml HBSS, becoming final conc. of 2.54 mM.

Time (min)	Radiation (gr)	N <sub>2</sub> + Drug	N <sub>2</sub>	O <sub>2</sub> + Drug	O <sub>2</sub>
9	18	20,000	10,000		
6	12	2000	1000		
3	6	1000	500		
0	0	200	200		
0	0	2		200	200
2	4			1000	1000
3	6			5000	5000
5	10			20000	20,000

Cells were allowed to attach for 2 1/2 hr, 3 ml of drug was added into 60 petri dishes, pumped for 1 hr, or plated in incubator for 1 hr, then put in 37°C water bath for 1 hr, total exposure time to drug was 2 hr.

Irradiated at 48 cm, at 20 rad inside chamber equals 200 rad/min.

Counts	N <sub>2</sub> + Drug	mean	SF	SF/PE	N <sub>2</sub>	mean	SF	SF/PE
1864	0 0 0	0	0	0	55.75. 61	63.7	0.006	0.01
1264	0 0 0	0	0	0	58.70. 33	53.7	0.054	0.11
664	1. 2. 0	1.	0.001	0.056	76. 63. 80	73	0.146	0.29
664	3. 7. 1.	3.7	0.018	(PE)	100. 112. 93	101.7	0.51	(PE)
O <sub>2</sub> + Drug		O <sub>2</sub>						
1064	27. 21. 26	24.7	0.004	0.008	74. 89. 78	80.3	0.004	0.005
664	95. 97. 67	86.3	0.02	0.049	287. 307. 312	302.7	0.061	0.078
464	40. 54. 46	46.7	0.05	0.267	239. 218. 219	225.3	0.225	0.29
0	36. 44. 25	35	0.175	(PE)	153. 142. 172	155.7	0.78	(PE)

## 2. Taxol and Taxol-metronidazole Toxicity experiments:

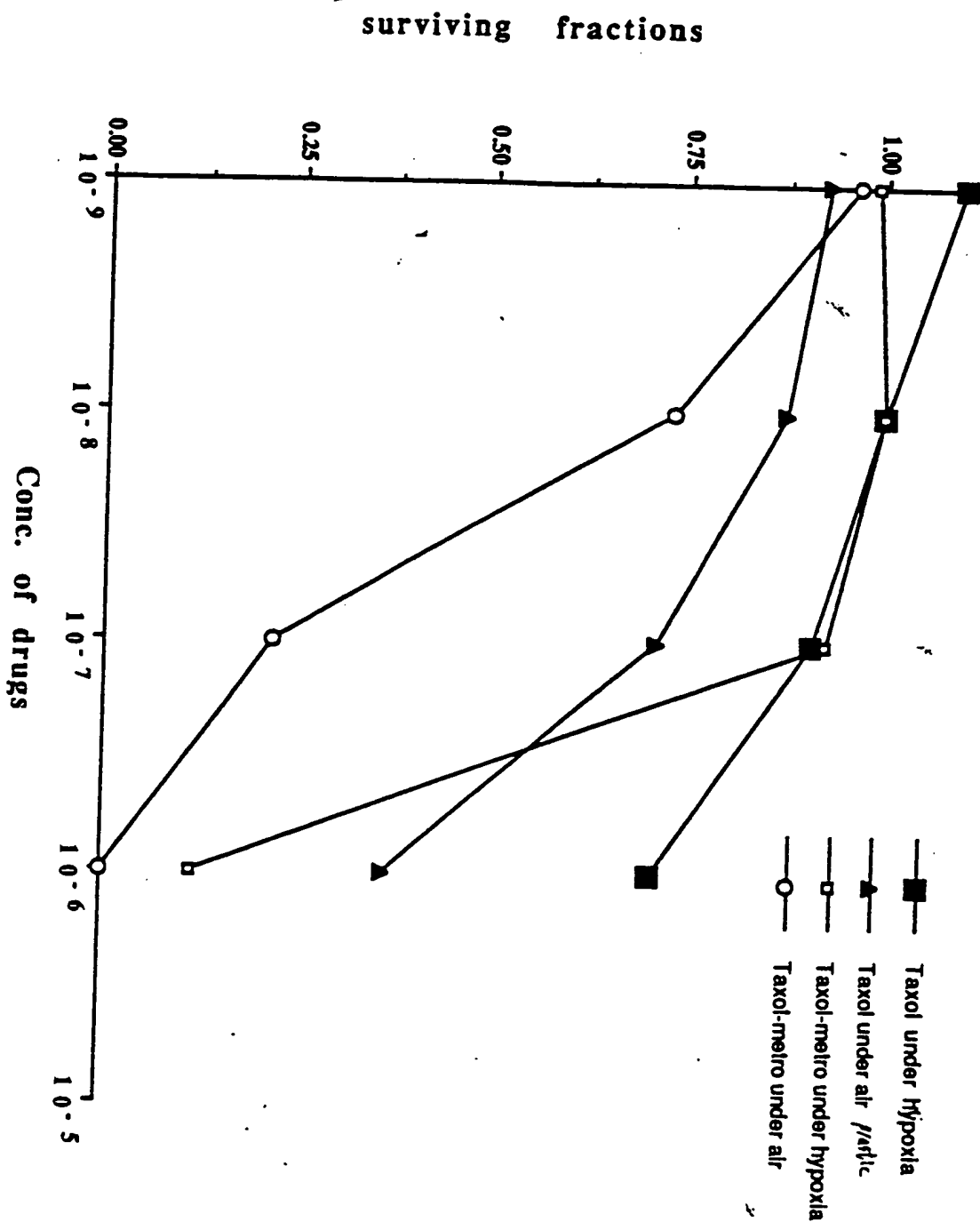
Drugs: Taxol:  $8.5 \mu\text{g} / 10 \text{ ml DMSC} = 1 \text{ mM}$   $\xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 2.075 \text{ ml} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-5} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-6} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-7} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-8}$

Taxol-metro:  $9 \mu\text{g} / 9 \text{ ml DMSC} = 1 \text{ mM}$   $\xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 2.075 \text{ ml} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-5} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-6} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-7} \xrightarrow{2 \text{ ml} + 10 \text{ ml DMSC}} 10^{-8}$

Cells were allowed to attach for 6h, drugs were added, pumped forth or plated in incubator foroxic groups, total drug exposure time was 24h. Each group had 2 parallel dishes, where 200 cells were plated for each of them.

Taxol V2	Count	mean	SF	SF/PE	O2 Count	mean	SF	SF/PE
T-9 2dish	141. 157	149	0.745	1.008	128. 145	141.5	0.7075	0.925
T-8 2dish	140. 132	136	0.68	1.011	128. 139	133.5	0.6675	0.873
T-7 2dish	131. 114. 122.5		0.6125	0.911	112. 106	109	0.545	0.712
T-6 2dish	47. 94. 95.5		0.4775	0.71	51. 60	55.5	0.2775	0.363
0 2dish	132. 137. 134.5		0.6775 (PE)		159. 147	153	0.765 (PE)	
Taxol-metro V2	Count	mean	SF	SF/PE	O2			
Tm-9 2dish	123. 143	133	0.665	0.989	148. 147	147.5	0.7375	0.964
Tm-8	121. 150	135.5	0.6775	1.00	112. 111	111.5	0.5575	0.729
Tm-7	122. 127	124.5	0.6225	0.926	35. 31	33	0.165	0.216
Tm-6	0. 31. 15.5		0.0775	0.15	0 0	0	0	0

## First Toxicity Experiment for Taxol &amp; Taxol-metro



# Radiosensitization of Taxol-M at Conc. of $2 \times 10^{-8} M$ .

Time	Radiation (Gy)	N <sub>2</sub> + Drug	N <sub>2</sub>	Time	Radiation (Gy)	S <sub>2</sub> + D	C <sub>2</sub>
7	18	20.000	10.000	4	8	10.000	10.000
6	12	5000	2000	3	6	5000	5000
3	6	1000	500	2	4	1000	1000
0	0	200	200	0	0	200	200

Drugs from Taxol-metro 1mM  $\rightarrow 0.025 \text{ ml} + 2.075 \text{ ml medium}$

Plated cell in dish put incubator 6hr then pump 1hr

$$1 \text{ ml} + 9 \text{ ml} = 10^{-6}$$

With chamber incubator 24 hr. Radiation

$$2 \text{ ml} + 18 \text{ ml} = 10^{-7}$$

incubator for ox.c

$$2 \text{ ml} + 8 \text{ ml} = 2 \times 10^{-8} \text{ Fresh dish add}$$

N <sub>2</sub> + Drug Count	mean	SF	S <sub>2</sub> /P <sub>2</sub>	N <sub>2</sub> Count	mean	SF	S <sub>2</sub> /P <sub>2</sub>	4ml
1867	66.69.99	78	0.0039	83.32.95	86.7	0.0087	0.011	1867
1267	306.291.29.7	298	0.0555	218.200.220	212.7	0.106	0.1399	1267
667	272.270.284	275.3	0.275	232.250.248	243.3	0.487	0.64	667
0	100.114.123	127	0.635 (PE)	162.146.148	152	0.76 (PE)		0

O<sub>2</sub> + Drug

C<sub>2</sub> Count

O <sub>2</sub> + Drug	mean	SF	S <sub>2</sub> /P <sub>2</sub>	C <sub>2</sub> Count	mean	SF	S <sub>2</sub> /P <sub>2</sub>	867
867	87.124.85	97.7	0.0098	188.199.194	193.7	0.019	0.035	867
667	150.146.149	148.3	0.03	400.395.393	396	0.079	0.104	667
467	148.126.134	136	0.136	333.353.356	343.3	0.347	0.457	467
0	88.90.89	89	0.045 (PE)	160.145.151	152	0.76 (PE)		0

# Radiosensitization of Taxol conc of $10^{-7}$

Drugs: Taxol 1mM  $\rightarrow$  0.025ml + 2.475ml =  $10^{-5}$   
(medium)

1ml + 9ml =  $10^{-6}$

10ml + 90ml =  $10^{-7}$

Time	Radiation (Gy)	N2+Drug	N2	Time	Radiation (Gy)	G2+Drug	G2
18	20.000	100000		8	10.000	100000	
12	5000	2000		6	5000	5000	
6	1000	500		4	1000	1000	
0	200	200		0	200	200	

Plated cell in dish incubator 6h then pump 1h with chamber incubator

24 hr then radiation incubator for 5X10

N2+Drug Count	mean	SF	SFE	N2 Count	mean	SF	SFE
1864 126.121.94	113.7	0.0057	0.0098	90.113.80	94.3	0.0074	0.0134
1264 370.274.359	267.7	0.0535	0.093	129.136.150	138.3	0.069	0.098
664 135.176.231	205.3	0.205	0.356	172.175.178	175	0.35	0.486
0 120.108.118	115.3	0.577 (PE)		146.141.136	141	0.705 (PE)	

G2+Drug	mean	SF	SFE	G2	mean	SF	SFE
864 215.214.220	216.3	0.0216	0.038	290.280.283	284.3	0.028	0.0414
664 338.335.372	278.3	0.0557	0.098	354.347.352	351	0.0702	0.102
464 133.139.141	137.6	0.138	0.243	350.286.254	263.3	0.263	0.383
0 118.114.108	113.3	0.567 (PE)		145.140.127	137.3	0.687 (PE)	

Radiosensitization of  $(\text{met})_2\text{N}(\text{CH}_3)_2\text{N}(\text{met})_2$  (4C)

Time	Radiation (Gy)	N <sub>2</sub> + Drug	N <sub>2</sub>	Time (hr)	Radiation (Gy)	N <sub>2</sub> + Drug	N <sub>2</sub>	T=
4'	18	20,000	10,000	4'	8	10,000	10,000	T=
6'	12	5000	2000	3' - 6		5000	5000	T=
3'	6	1000	500	2'	4	1000	1000	T=
0		200	200	0		200	200	T=

Plated cell in dish incubation 2.5h then add Drug pump 1h. then put waterbath

37°C 1hr <sup>then</sup> radiationoxic in incubator

Drug:  $(\text{met})_2\text{N}(\text{CH}_3)_2\text{N}(\text{met})_2$  : 2.8.2 mg / 1 ml water. FW. 686.18 = 0.679 mM

7 days later, stained, counted.

N <sub>2</sub> + Drug Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE	T=
1864	0	0	0	82.44.46	74	0.0074	0.02	T=
1264	0	0	0	125.129.120	124.7	0.062	0.168	T=
664	36.8.9	17.7	0.0035	0.024	136.132.90	119.3	0.124	0.65
0	43.23.25	30.3	0.15 (PE)	46.44.82	74	0.37 (PE)		

02 + Drug

N <sub>2</sub> + Drug	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE	T=
864	704.219.204	209	0.0209	0.038	227.224.232	227.7	0.023	0.034
664	296.318.305	306.3	0.061	0.11	328.314.311	317.7	0.064	0.085
464	192.168.187	182.3	0.182	0.33	207.213.230	216.7	0.247	0.323
0	105.120.109	111.3	0.557 (PE)	118.130.155	134.3	0.67 (PE)		

## Taxol and Taxol-mithrebenzine toxicity

T=Taxol	N <sub>2</sub> Count	mean	SE	SE = 22 Count	mean	SE	SE/PE
T-5	71.74.95	82.67	0.41	0.74	4.5.1	5	0.025
T-6	82.25.71	76	0.38	0.63	25.25.19	23	0.115
T-7	94.93.82	89.7	0.428	0.80	80.63.74	73.7	0.37
T-8	128.110.118	118.7	0.59	1.06	141.115.132	129.3	0.65
T-9	105.106.126	112.3	0.56	1.007	132.126.119	125.7	0.63
TNB = Taxol-mithrebenzine							
TNB-5	0.0.0	0	0	0	0.0.0	0	0
TNB-6	39.49.52	46.7	0.23	0.42	2.2.0	1.3	0.007
TNB-7	91.81.96	89.3	0.447	0.80	40.19.32	30.3	0.115
TNB-8	84.98.96	92.7	0.46	0.83	29.29.57	38.3	0.19
TNB-9	102.92.102.	98.7	0.49	0.88	66.70.50	62	0.31
Q	110.111.114	111.7	0.558 (PE)	122.119.128	123	0.615 (PE)	0.50

Each dish add 250 cell. put cell in dish incubator 6 hr. then add drug pump/hr after pump. With chamber incubator 24 hr. Oxic in incubator.

Drug Taxol from 1 mM  $\rightarrow$  0.3 ml + 29.7 ml =  $10^{-5}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-6}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-7}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-8}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-9}$

TNB 1 mM  $\rightarrow$  0.3 ml + 29.7 ml =  $10^{-5}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-6}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-7}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-8}$   
 $\downarrow$   
 3 ml + 27 ml =  $10^{-9}$

## Radiosensitization of DPTM=3C

Time	RT (Gy)	<sup>(N<sub>2</sub>)</sup> Hypoxic + Drug	N <sub>2</sub>	Time	RT (Gy)	O <sub>2</sub> + Drug	O <sub>2</sub>
4 4'5"	18	20,000	10,000	4 1/2"	8	10,000	10,000
6 6'0"	12	5000	2000	3 1/2"	6	5000	5000
3 3'2"	6	1000	500	2 1"	4	1000	1000
	0	200	200	0	0	200	200

plated cell in dish incubator 2.5 hr. then add drug pump 1 hr. after put Waterbath 37°C 1 hr <sup>then</sup> irradiation sic in incubator.

Drug from DPTM=3C

$$10.9 \text{ ml} + 20 \text{ ml} = 1 \text{ mM}$$

$$8 \text{ ml} + 72 \text{ ml} = 0.1 \text{ mM}$$

(Drug) (1055)

## Count

8 N <sub>2</sub> + Drug Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
12 8'4" 78.53.62	64.3	0.003	0.0066	123. 102.72.	99	0.0094	0.017
6 12'4" 98.88.113	49.7	0.02	0.0407	71. 80. 72.	74.3	0.037	0.073
16 4" 118.126.135	126.3	0.126	0.258	132. 85. 79	98.7	0.20	0.39
0 102.42.98	97.3	0.49 (PE)		114. 84. 110	102.7	0.51 (PE)	

86.

16 O <sub>2</sub> + Drug	mean	SF	SF/PE	O <sub>2</sub>	mean	SF	SF/PE
14 8'4" 89.92.107	96	0.0096	0.0192	162. 152. 149.	154.3	0.015	0.027
0 6'4" 103.170.170	161	0.0322	0.064	228. 235. 231	231.3	0.046	0.08
16 4" 120.133.129	127.3	0.127	0.25	166. 141. 165	157.3	0.457	0.27
0 102.95.105	100.7	0.50 (PE)		104. 121. 121	115.3	0.58 (PE)	

## DBTM + Radiation

DBTM = 4C

from DBTM 9.697 mg/ml

0.54 ml + 74.46 ml = 0.1 mM

RT (min)	N <sub>2</sub> + D	N <sub>2</sub>	Time (min)	RT (min)	N <sub>2</sub> + D
18	20,000	10,000	8	5000	5000
120	5000	2000	-6	2000	2000
6	1000	500	4	1000	1000
0	200	200	0	200	200

plated cell in dish 3.5 hr incubator. then add drug pump 1 hr. after pump put water bath 37°C 1 hr. <sup>then</sup> radiation. after wash off the drug. add new medium keep in incubator for 8 hrs.

N <sub>2</sub> + Drug Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
46.35.35	38.7	0.0019	0.006	101.91.30	74	0.0074	0.053
47.81.127	85	0.017	0.053	83.49.51	61	0.0305	0.22
77.83.124	94.7	0.045	0.3	29.22.82	44.3	0.09	0.63
73.32.88	64.3	0.32 (PE)		42.25.15	27.3	0.14 (PE)	

O<sub>2</sub> + DrugO<sub>2</sub>

O <sub>2</sub> + Drug	mean	SF	SF/PE	O <sub>2</sub>	mean	SF	SF/PE
84.53.73	70	0.014	0.026	104.97.87	96	0.019	0.03
110.129.116	118.3	0.059	0.106	150.141.151	147.3	0.074	0.12
164.154.161	159.7	0.16	0.29	159.173.176	169.3	0.17	0.28
120.110.106	112	0.56 (PE)		120.136.110	122	0.61 (PE)	

CTM=5C

## Toxicity Experiment

C 1. 101.48

H<sub>2</sub> 0.23

C 2. 110.106

19.5

C 3. 108.124

6.12

120.136.150

0.61 (PE)

42.25.15

0.14 (PE)

plated cell in dish. 3.5 hr incubator. Each dish add 200 cells.

add DMSO pump 1 hr. then waterbath 1 hr.

5C = 0.15 ml + 14.85 ml (HBSS) = 1 mM 5C1.

↓  
1.5 ml + 13.5 ml HBSS = 0.1 mM 5C2.

↓  
1.5 ml + 13.5 ml HBSS = 0.01 mM 5C3.

CTM = 5C + Radiation

Radiation (Gy) N <sub>2</sub> + Drug	N <sub>2</sub>	Radiation (Gy) O <sub>2</sub> + Drug	O <sub>2</sub>
864 20.000	10.000	864 10.000	5000
1264 5000	2000	6 2000	1000
664 1000	500	4 1000	500
0 200	200	0 200	200

Drug: from CPM = 5C  $0.15 \text{ ml} + 14.85 \text{ ml} = 1 \text{ ml}$

$0.025 \text{ ml} + 25 \text{ ml} = 0.1 \text{ ml}$   
(x ml) HBSS

plated cell in dish 2.5 hr. incubator. then add drug pump hr. then

waterbath 1 hr 37°C, then radiation.

N <sub>2</sub> + Drug Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
38.44.14	33.7	0.0017	0.003	158.185.164	172.3	0.017	0.025
1264 62.72.94	76	0.0152	0.028	157.127.119	134.3	0.067	0.099
664 159.143.72	124.7	0.12	0.23	154.155.173	160.7	0.32	0.47
0 109.108.109	108.7	0.54 (PE)		136.132.140	136	0.68 (PE)	
O <sub>2</sub> + Drug				O <sub>2</sub>			
864 198.232.215	215	0.0215	0.032	99.113.95	102.3	0.02	0.031
664 140.156.129	141.7	0.071	0.106	67.64.80	70.3	0.07	0.107
464 158.181.150	163	0.163	0.24	113.104.99	105.3	0.21	0.319
0 138.139.124	133.7	0.67 (PE)		144.124.128	132	0.66 (PE)	

## Taxol and Taxol-nitrobenzine toxicity

	N <sub>2</sub>	Count	mean	SF	SF/PE	O <sub>2</sub>	Count	mean	SF	SF/PE
T-5	dish	43.52	47.5	0.2375	0.62	2dishes	5.10	7.5	0.0375	0.07
T-6	"	46.53	49.5	0.2475	0.647	"	13.2	19.5	0.0975	0.17
T-7	"	95.93	94	0.47	1.23	"	72.5	61	0.305	0.53
T-8	"	74.73	73.5	0.3675	0.96	"	127.121	124	0.62	1.08
T-9	"	80.80	80	0.4	1.05	"	46.114	105	0.535	0.91
0	"	74.74	76.5	0.3825 (PE)		"	135.105	115	0.575 (PE)	
TNB-5	"	0 0	0	0		"	0 0	0	0	0
TNB-6	"	33.20	26.5	0.1325	0.35	"	1.3	2	0.01	0.07
TNB-7	"	63.77	73	0.2825	0.74	"	17.25	24.0	0.105	0.48
TNB-8	"	69.77	73	0.365	0.95	"	55.58	56.5	0.28	0.49
TNB-9	"	81.81	81	0.405	1.06	"	90.84	87	0.435	0.76

Each dish add 200 cell. put incubator 6hr. then add drug. Hypoxic pump 1hr. then with chamber put incubator 24hr. or incubator 24hr. after wash off the drug.

$$\text{Taxol: } 0.2 + 19.8 = 10^{-5}$$

$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-6}$$

$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-7}$$

$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-8}$$

$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-9}$$

$$\text{TNB: } 0.2 + 19.8\text{ml} = 10^{-5}$$

$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-6}$$

$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-7}$$

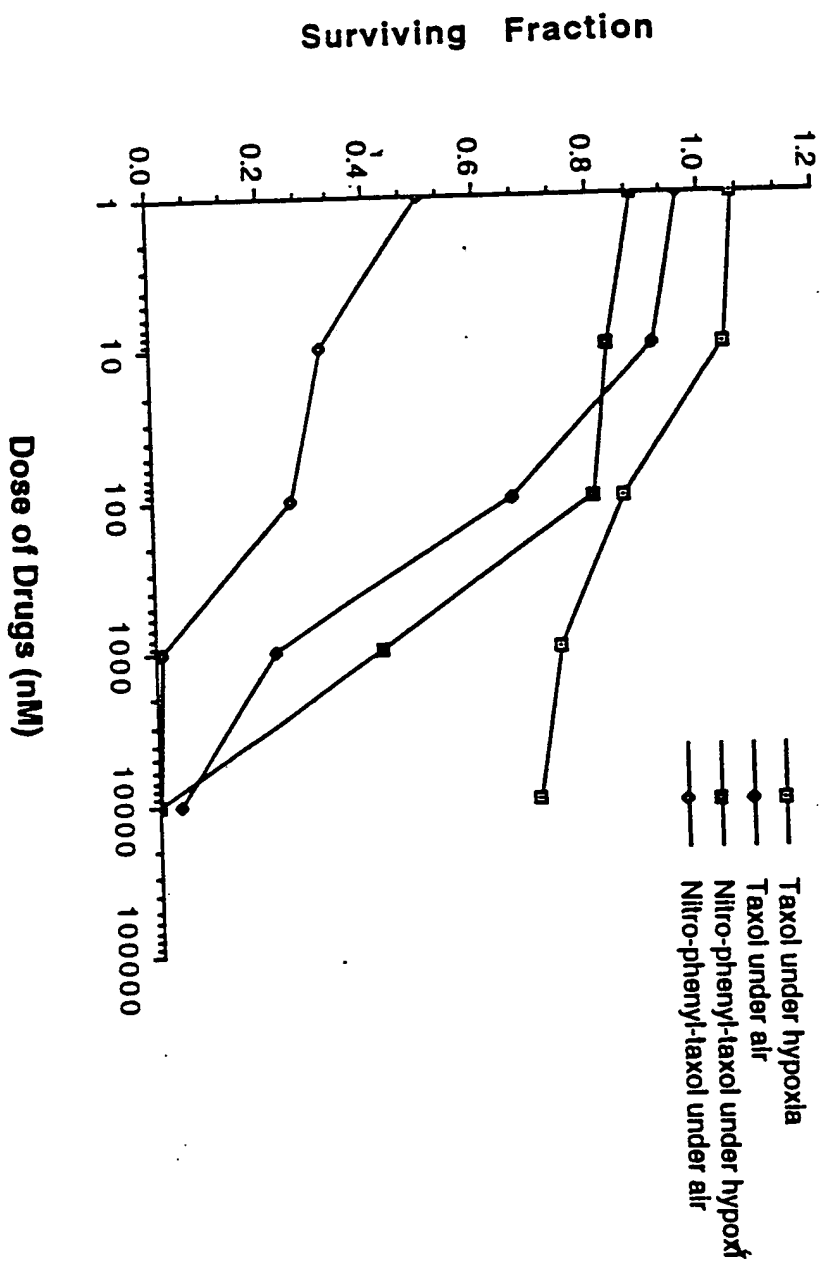
$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-8}$$

$$\downarrow$$

$$2\text{ml} + 18\text{ml} = 10^{-9}$$

### Chemotherapeutic activities of Taxol & Nitro-phenyl-taxol against CHO Cells



DBTM = 4C + Radiation

DBTM = 0.1 mm

RT Time (Gy) N <sub>2</sub> + Drug	N <sub>2</sub>	RT (Gy) O <sub>2</sub> + Drug	O <sub>2</sub>
8 20.800	10000	8 5000	5000
12 5000	7000	6 2000	1000
6 1000	500	4 1000	500
0 200	200	0 200	200

Drug: from 9.697 mm.

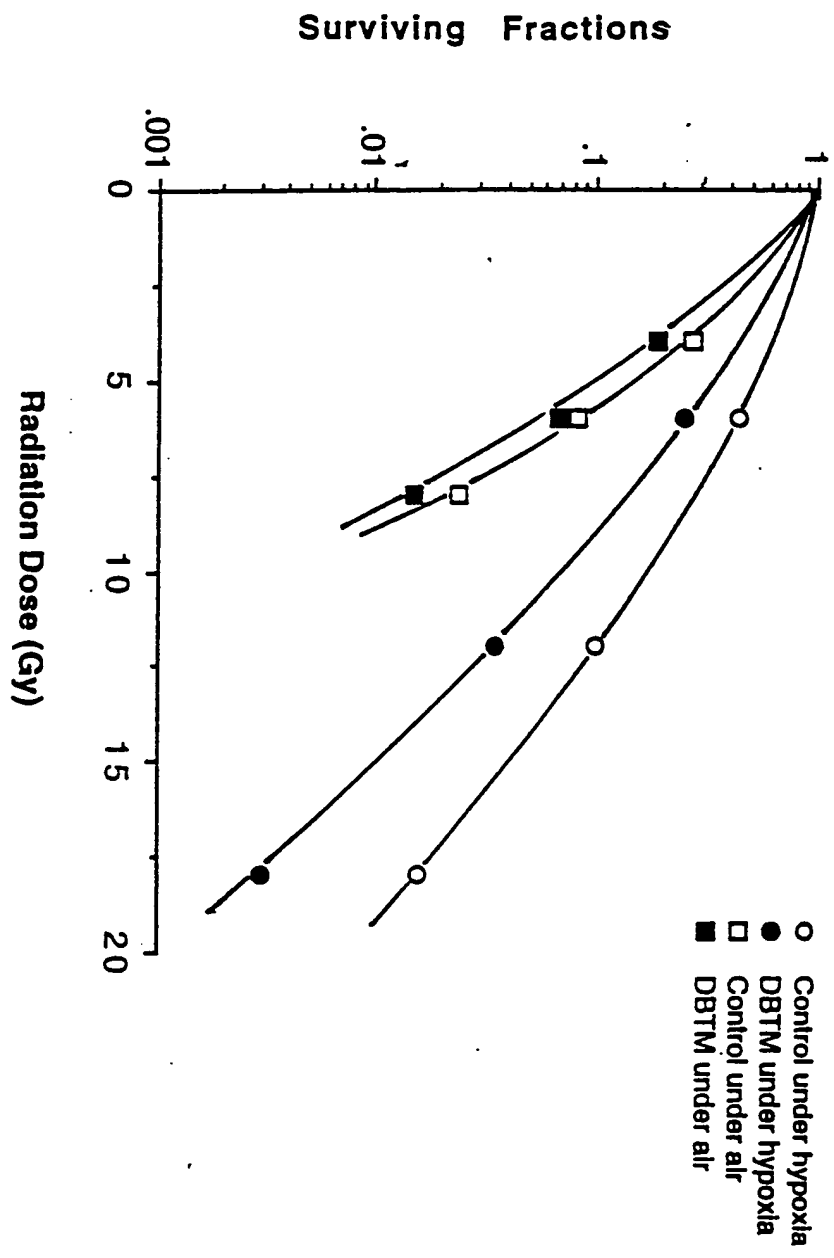
$$1.62 \text{ ml} + 73.4 = 0.2 \text{ mm.}$$

Plated cell in dish 2.5 hr. incubator. then add drug pump 1 hr. then put waterbath 37°C  
1 hr. O<sub>2</sub> in incubator. After Radiation

N <sub>2</sub> + 12 Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
9Gy 30.38.29	32.3	0.0016	0.0029	83.86.94	87.7	0.0088	0.06
12Gy 106.43.88	45.7	0.019	0.035	122.109.106	112.3	0.056	0.1
6Gy 119.149.144	137.3	0.157	0.25	131.126.113	123.3	0.25	0.44
0 45.110.98	107.7	0.54 (PE)		120.103.115	112.3	0.56 (PE)	

O <sub>2</sub> + D count				O <sub>2</sub> Count			
8Gy 43.39.43	41.7	0.008	0.015	61. 61.58	60	0.012	0.024
6Gy 68.79.72	73	0.0365	0.068	40. 39.49	42.7	0.043	0.084
4Gy 105.101.97	101	0.101	0.19	58. 74.75	69	0.138	0.27
0 103.113.110	108.7	0.54 (PE)		115. 95.94	101.3	0.51 (PE)	

# Radiosensitizing effects of DBTM (0.1 mM) on CHO Cells



CTM = 5C + Radiation				CTM = 0.1 mm	
Radiation (Gy)	N <sub>2</sub> + Drug	N <sub>2</sub>	RT (Gy)	G <sub>2</sub> + Drug	G <sub>2</sub>
18	20,000	10,000	8	10,000	5000
12	5000	2000	6	2000	1000
6	1000	500	4	1000	500
0	200	200	0	200	200%

Drug: from 100 mm.

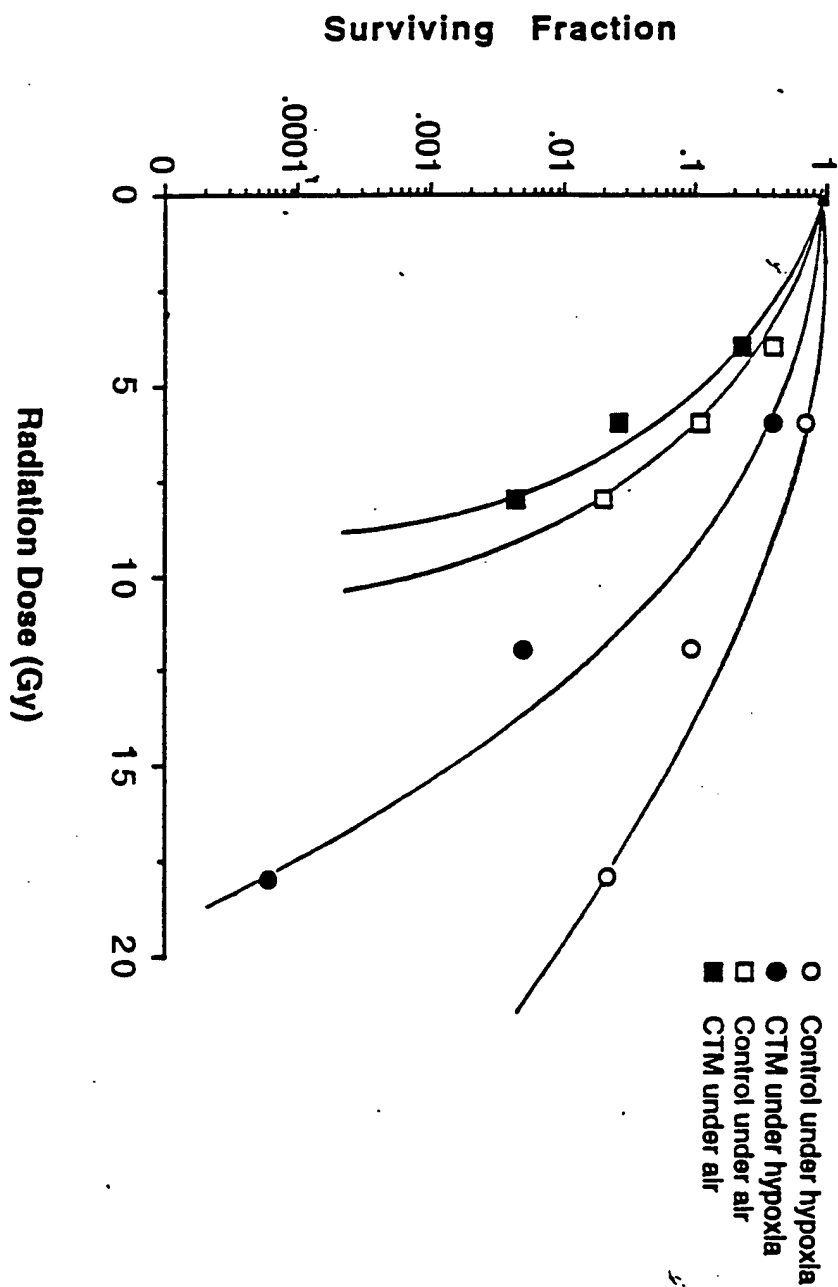
Drug: from 100 mM.

$$0.076 \text{ mol} + 75 \text{ mol} = 0.1 \text{ mol}$$

plated cell in dish incubator 3.5 hr <sup>(HBSS)</sup> then add drug pump/hr. after put waterbath 37°C 1 hr. radiation optic working in incubator.

37°C 1 hr. Radiation					oxic working in incubator				
N <sub>2</sub> + Drug Count		mean	SF	SF/PE	N <sub>2</sub> Count		mean	SF	SF/PE
18Gy	1. 0. 0.	0.3	0.00002	0.00006	114. 109. 95	106	0.0106	0.022	
12Gy	6. 7. 8	7	0.0016	0.005	83. 113. 83	93	0.0465	0.097	
6Gy	101. 104. 123	109.3	0.109	0.4	166. 162. 166	164.7	0.33	0.69	
0	68. 45. 48	53.7	0.27 (PE)		100. 88. 99	95.7	0.48 (PE)		
O <sub>2</sub> + Drug Count					O <sub>2</sub> Count				
8Gy	22. 18. 16	18.7	0.0019	0.0043	47. 42. 51	46.7	0.009	0.02	
6Gy	27. 16. 25	22.7	0.011	0.026	35. 44. 65	48	0.048	0.109	
4Gy	78. 107. 115	100	0.1	0.23	88. 87. 90	88.3	0.18	0.4	
0	88. 108. 63	86.3	0.43 (PE)		94. 82. 89	88.3	0.44 (PE)		

## Radiosensitizing Effects of CTM (0.1 mM) on CHO Cells



CTM = 5C + Radiation

CTM = 0.1 mM

Rad	RT	N <sub>2</sub> + Drug	N <sub>2</sub>	RT (Ct)	O <sub>2</sub> + Drug	O <sub>2</sub>
L	18	20.000	10.000	8	5000	5000
12	12	5000	2000	-6	1000	1000
6	6	1000	500	4	500	500
0	0	200	200	0	200	200

Drug: from 100 mM

$$0.076 \text{ ml} + 75 \text{ ml} = 0.1 \text{ mM}$$

plated cell in dish incubator 3 hr. add drug pump 1 hr. then put water bath 37°C 1 hr.  
then radiation oxic in incubator.

	$N_2 + \text{Drug Count}$	mean	SF	SF/PE	$N_2 \text{ Count}$	mean	SF	SF/PE
18G	1. 7. 2.	3.3	0.00017	0.0003	140. 145. 166	150	0.005	0.025
12G	25. 15. 40	26.7	0.0053	0.01	151. 145. 121	139	0.0695	0.114
6G	134. 97. 106	112.3	0.112	0.26	144. 158. 158	153.3	0.31	0.5
0	99. 110. 104	104.3	0.52 (PE)		123. 110. 132	121.7	0.608 (PE)	

	O <sub>2</sub> + Drug Count				O <sub>2</sub> Count			
8G	8G	98. 118. 104	106.7	0.02 0.03	121. 117. 107	115	0.023	0.03
6G	6G	81. 58. 80	73	0.073 0.114	61. 76. 57	64.7	0.065	0.095
4G	4G	126. 104. 107	112.3	0.225 0.35	122. 121. 111	118	0.236	0.35
0	0	124. 140. 121	128.3	0.64 (PE)	138. 135. 135	136	0.68 (PE)	

## Taxol and Taxol nitrobenzine toxicity

N <sub>2</sub>	mean	SF	SF/PE	O <sub>2</sub>	mean	SF	SF/PE
T-5	12.16			7.5			
T-6	29.21			9.4			
T-7	74.105			80.70			
T-8	79.74			147.144			
T-9	111.89			117.127			
0	120.106			130.109			
TNB-5	0			0			
TNB-6	33.33			1.4			
TNB-7	81.73			24.36			
TNB-8	84.44			56.82			
TNB-9	90.120			85.53			

T = Taxol

TNB = Taxol nitrobenzine

Each dish plated 200 cells in incubator 6 hr. then add drug hypoxic pump 1 hr.  
then with chamber put incubator 24 hr. or add drug incubator 24 hr.

Drug from: Taxol: 0.2 ml + 19.8 ml =  $10^{-5}$ 

$$\downarrow \text{(medium)}$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-6}$$

$$\downarrow$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-7}$$

$$\downarrow$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-8}$$

$$\downarrow$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-9}$$
TNB: 22 ml + 19.8 ml =  $10^{-5}$ 

$$\downarrow$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-6}$$

$$\downarrow$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-7}$$

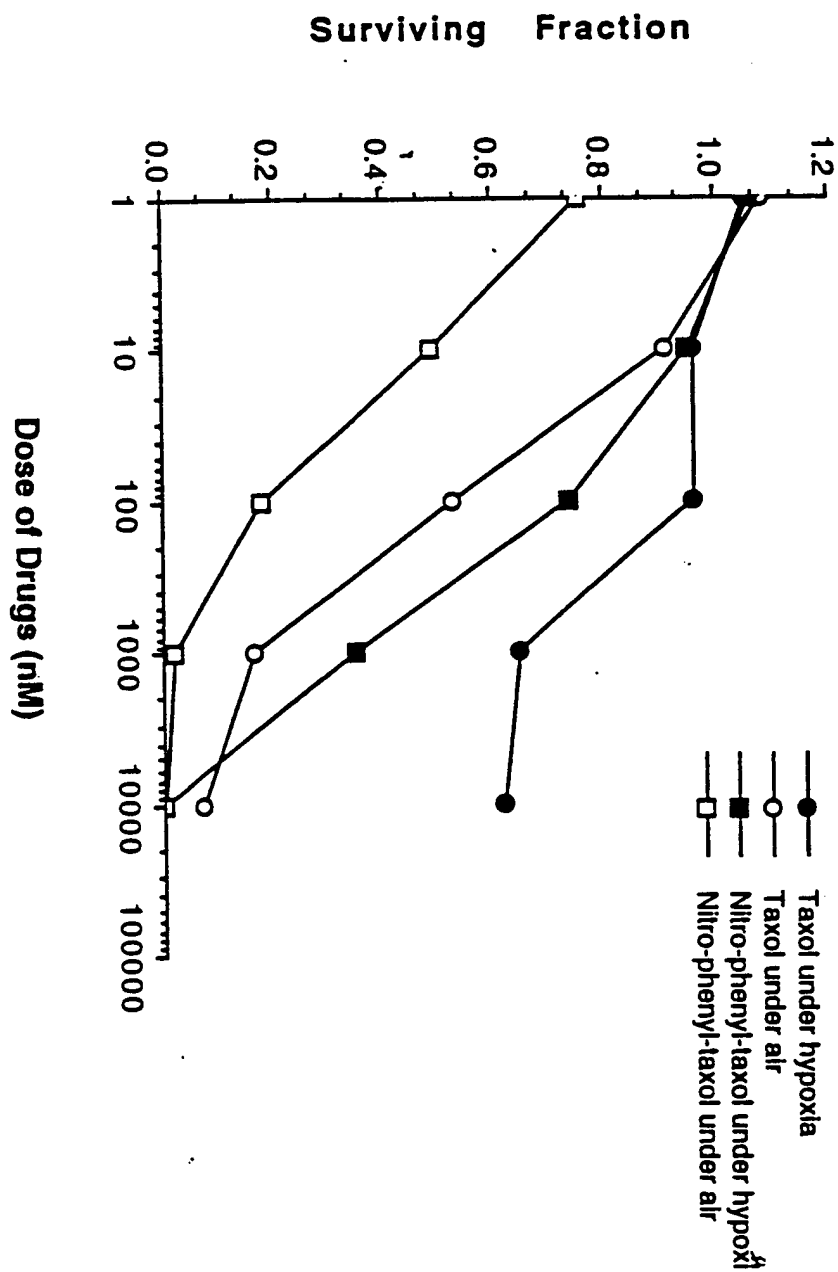
$$\downarrow$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-8}$$

$$\downarrow$$

$$2 \text{ ml} + 18 \text{ ml} = 10^{-9}$$

### Chemotherapeutic Activities of Taxol & Nitro-phenyl-taxol against CHO Cells



## Toxol-metry + Radiation

Calorimeter Radiation						
RT (Time)	RT (hr)	N <sub>2</sub> + D <sub>2</sub> O <sub>2</sub>	N <sub>2</sub>	RT (hr)	O <sub>2</sub> + D <sub>2</sub> O <sub>2</sub>	O <sub>2</sub>
	18	22.600	10.000	3	5000	2000
	12	5000	2000	6	2000	1000
	6	1000	500	4	1000	500
	0	200	200	0	200	200

Plated cell in dish incubator 6 hr then add drug pump 1 hr etc in incubator.  
after pump with chamber put incubator 24 hr then radiation.

Drug Toxol-metry 1mm.

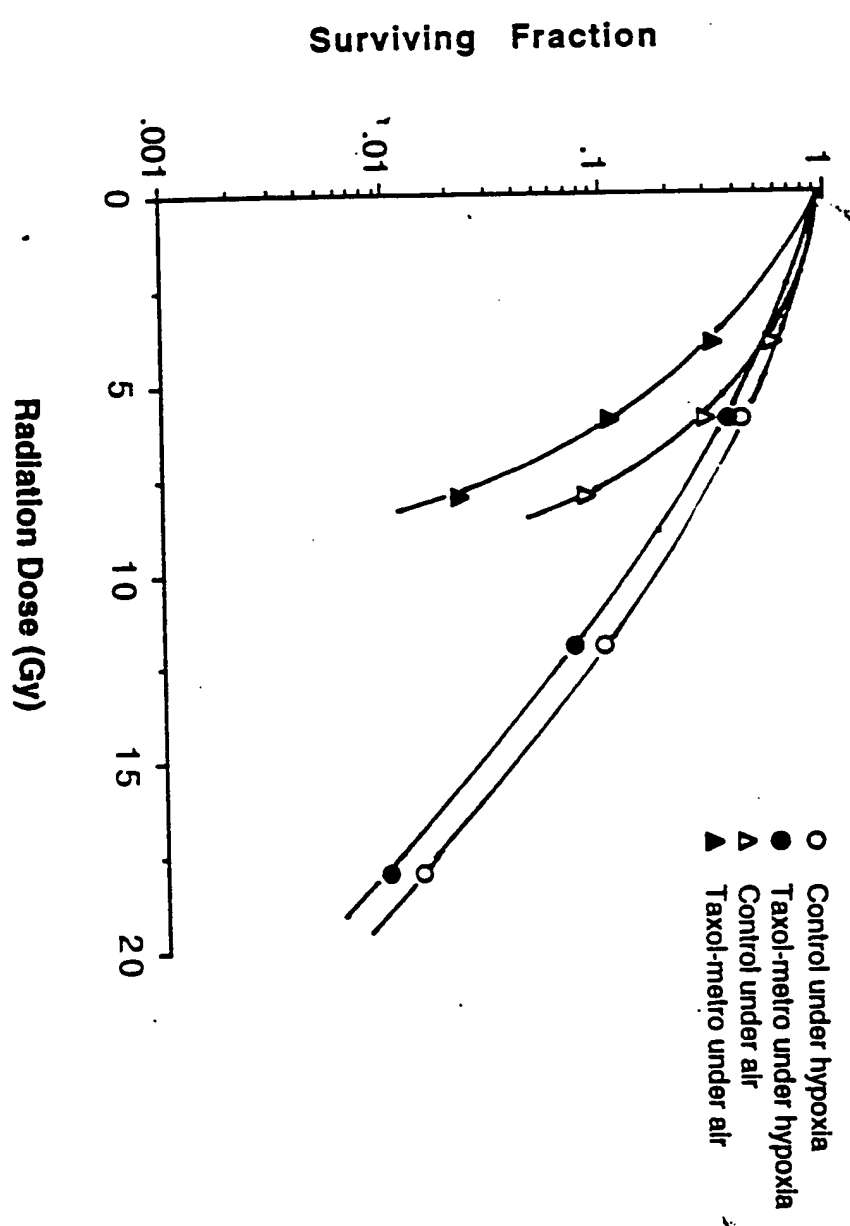
$$20 \mu\text{L} + 1.48 \mu\text{L} = 10^{-5}$$

↓ (medium)

$$10 \mu\text{L} + 10 \mu\text{L} = 10^{-6} \rightarrow 10 \mu\text{L} + 90 \mu\text{L} = 10^{-7}$$

N <sub>2</sub> + D <sub>2</sub> O <sub>2</sub> Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
110. 139. 115	121.3	0.006	0.01	118. 96. 84	99.3	0.0099	0.014
216. 227. 209	217.3	0.043	0.076	129. 184. 57	143.3	0.072	0.1
178. 239. 229	215.3	0.215	0.385	152. 167. 158	152.3	0.3	0.429
126. 103. 126	118.3	0.59 (PE)		139. 147. 138	141.3	0.71 (PE)	
O <sub>2</sub> + D <sub>2</sub> O <sub>2</sub> Count	mean	SF	SF/PE	O <sub>2</sub> Count	mean	SF	SF/PE
12. 16. 12.	13.3	0.003	0.022	127. 129. 128	121.3	0.06	0.085
25. 27. 30	27.3	0.044	0.11	214. 207. 226	215.7	0.216	0.3
31. 35. 50	38.7	0.079	0.52	209. 216. 217	214	0.428	0.599
24. 27. 23	24.7	0.12 (PE)		143. 138. 148	143	0.715 (PE)	

# Radiosensitization of Taxol-metro (100nM) on CHO Cells (1st exp)



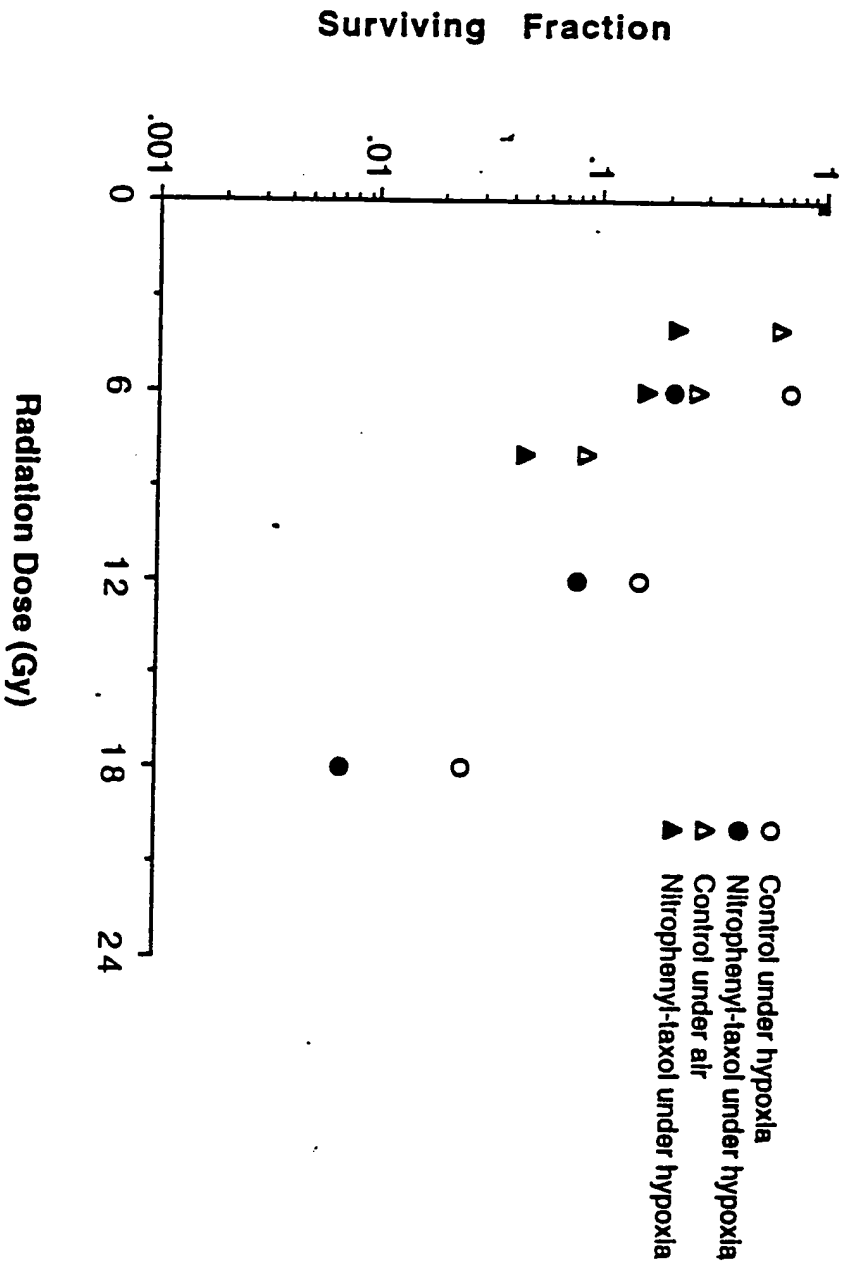
7.4% - nitrogenase + Radiation			T.V.B $10^{-7}$ ml		
(RT)	N <sub>2</sub> + D <sub>2</sub>	N <sub>2</sub>	(RT)	C <sub>2</sub> + D <sub>2</sub>	C <sub>2</sub>
18	2000	10000	8	5000	2000
12	5000	2000	6	2000	1000
6	1000	500	4	1000	500
0	200	200	0	200	200

plated cell in dish 5.5 hr then add drug pump 1 hr. after pump with chamber put incubator 24 hr or 10 incubator. then radiation.

Drug: from T.V.B  $10^{-7}$   $\rightarrow$  0.22 ml + 1.48 ml =  $10^{-5}$   $\rightarrow$  1 ml + 9 ml =  $10^{-6}$   $\rightarrow$  10 ml + 90 ml =  $10^{-7}$   
(medium)

N <sub>2</sub> + D <sub>2</sub> Count	mean	SF	SF/PE	RT Count	mean	SF	SF/PE
60 79 65	68	0.0034	0.007	152.183.169	168	0.0168	0.025
120 241.170.220	210.3	0.042	0.082	204.189.221	204.7	0.102	0.153
60 115.113.99	104	0.109	0.21	254.226.226	235.3	0.47	0.7
0 99.98.108	101.7	0.51 (PE)		130.129.105	134.7	0.67 (PE)	
C <sub>2</sub> + D <sub>2</sub> Count				C <sub>2</sub> Count			
80 50.45.60	51.7	0.01	0.046	121.121.120	120.7	0.06	0.087
60 72.71.70	71	0.0355	0.16	176.167.191	184.7	0.18	0.27
40 42.56.53	50.3	0.05	0.22	211.216.223	216.7	0.43	0.63
0 49.44.42	45	0.225 (PE)		129.145.135	137.3	0.69 (PE)	

## Radiosensitizing Effects of Nitro-phenyl-taxol (100 nM) on CHO Cells



$T=10^{-5}$ 

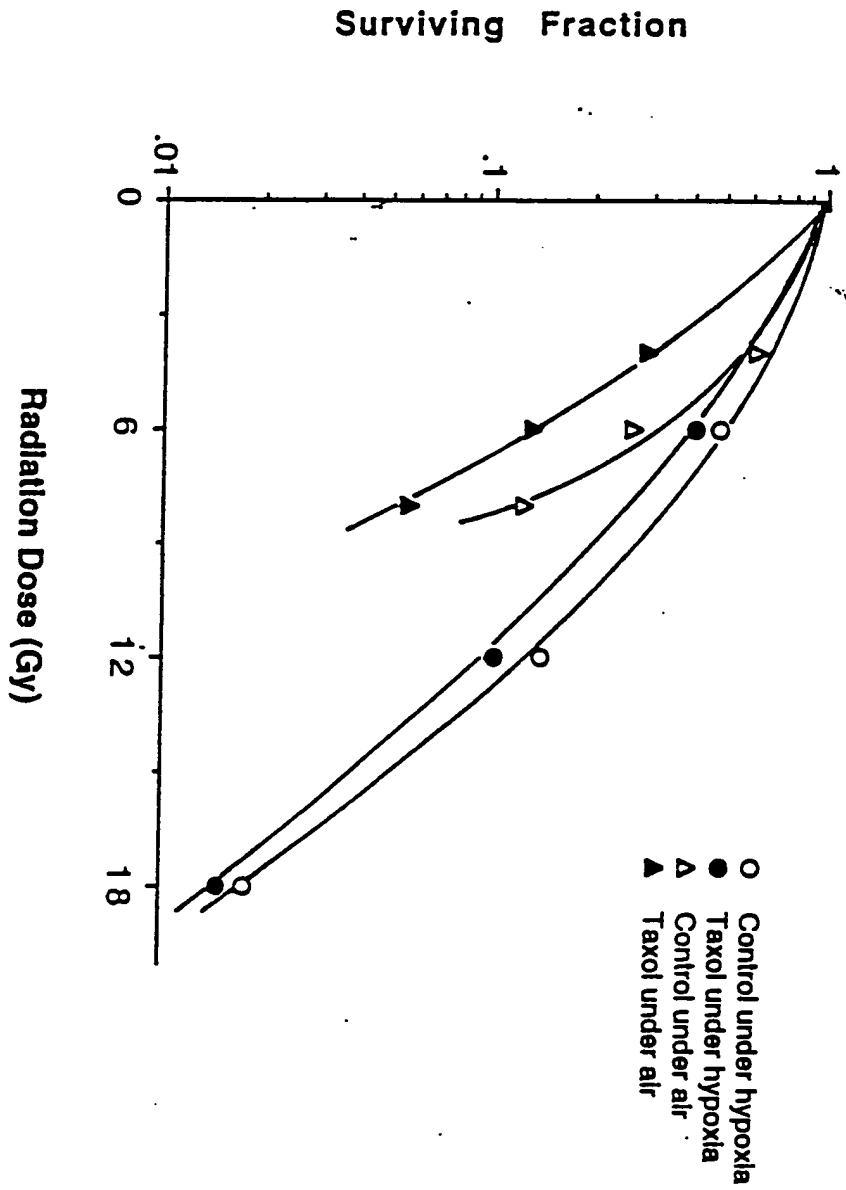
RT	Taxol + Radiation	Hypoxic RT	OTIC + b. 2%	OTIC
18	20.000	10.000	8	5000
12	5000	2000	6	2000
6	1000	500	4	1000
0	200	200	0	200

plated cell in dish for incubator. then add drug pump 1 hr. after pump with chamber incubator 24 hr. then Radiation OTIC in incubator.

Drug: from Taxol  $10^{-3}$   $0.5 \text{ ml} + 49.5 \text{ ml} = 10^{-5}$   
 $\downarrow$  (medium)  
 $5 \text{ ml} + 50 \text{ ml} = 10^{-6}$

$N_2$ + Drug Count	mean	BF	SF/PE	$N_2$ Count	mean	SF	SF/PE
80% 138. 110. 1127	125	0.006	0.015	105. 170. 90	108.3	0.011	0.018
120% 205. 209. 197	220.3	0.044	0.102	174. 161. 172	169	0.085	0.143
60% 49. 178. 194	173.7	0.174	0.404	148. 141. 137	142	0.284	0.481
0 85. 102. 70	85.7	0.03	(PE)				

$O_2$ + Drug Count	mean	BF	SF/PE	$O_2$ Count	mean	SF	SF/PE
80% 25. 26. 32	34.3	0.007	0.057	146. 161. 148	151.7	0.076	0.124
60% 39. 35. 22	32	0.016	0.133	172. 159. 154	161.7	0.162	0.265
40% 38. 39. 27	34.7	0.035	0.289	191. 186. 194	190.3	0.381	0.624
0 26. 18. 29	24.3	0.12	(PE)	119. 116. 133	122.7	0.61	(PE)

Radiosensitizing Effects of Taxol (5  $\mu$ M) on CHO Cells

## SC PLDR Experiment

Drug = 2.1 mM  
RT = 300 f

0 hours RT 5000

2 hr RT 2000

2 hr RT + Drug 2000

2 hr Drug 200

4 hr RT 1000

4 hr RT + Drug 1000

4 hr Drug 200

6 hr RT 500

6 hr RT + Drug 500

6 hr Drug 200

Plated cells each dish  $2 \times 10^5$  3 dishes later (total 10 dishes) take 3 dishes add drug 7 dishes  
8 Gy of x-ray radiation 4: after RT 3 dish add drug. 3 dish no drug. put incubator. 2 hr. 4 hr. 6 hr.

trypsin then plated cell in dish. incubator 8 day.

Drug: from 100 mM.

0.02 ml + 3.8 ml = 4 ml. each dish add 0.5 ml  
(HES) 0 + 2 ml old medium. total 2.5 ml

Count	mean	sf	sf/PE
2 hr 122.118.125	124.7	0.61	0.83
4 hr 134.113.134	127	0.635	0.87
6 hr 129.134.129	130.7	0.65	0.89
0 154.136.147	145.7	0.73 (PE)	

## SC and Metor Toxicity

N <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>	O <sub>2</sub>	
C1 3dishes	3dishes	M1 3dishes	3dishes	SC1 = 1mM
C2 3dishes	"	M2 "	"	SC2 = 0.1mM
C3 3dishes	"	M3 "	"	SC3 = 0.01mM
				M1 = Metor 20mM
				M2 = Metor 10mM
				M3 = Metor 1mM

Plated cell in dish incubator 3.5 hr. then add drug oric and Hypoxic pump 1hr. after pump put waterbath 37°C 1hr. then wash out drug. add new medium. incubation 7 days.

Drug from SC 100mM  $\rightarrow 0.2\text{ml} + 19.8\text{ml} = 1\text{mM}$   
 $\downarrow$  (HBSS)  
 Metor:  $136\text{mg}/40\text{ml HBSS} = 3.4\text{mM}$   
 $\downarrow$   
 $1\text{ml} + 19\text{ml} = 10\text{mM}$   
 $\downarrow$   
 $2\text{ml} + 18\text{ml} = 1\text{mM}$   
 $2\text{ml} + 18\text{ml} = 0.1\text{mM}$   
 $2\text{ml} + 18\text{ml} = 0.01\text{mM}$

N <sub>2</sub> Count	mean	SF	SF/PE	O <sub>2</sub> Count	mean	SF	SF/PE
1mM SC1 2000				000			
0.1mM SC2 124.144.124	129.7	0.65	1.137	133.116.138	129	0.645	1.008
0.01mM SC3 128.127.129	128	0.64	1.123	129.145.137	137	0.685	1.07
0 Control 115.112.114	113.7	0.57 (PE)					
20mM M1 117.99.111	109	0.545	0.46	130.146.103	126.3	0.63	0.99
10mM M2 128.107.118	117.7	0.59	1.03	136.133.125	131.3	0.66	1.026
1mM M3 96.135.123	118	0.59	1.035	133.145.128	135.3	0.68	1.057

Taxol-meter + Radiation			CHO cell	Drug = $10^{-7}$	
T	$N_2 + \text{Drug}$	$A_{420}$	RT (60s)	$O_2 + \text{Drug}$	$O_2$
1	20.000	10.000	8	5000	2000
2	2000	2000	6	2000	500
3	500	500	4	1000	200
4	200	200	0	200	200

plate cell in dish 3 hr. incubator. then add drug.  $O_2$  in incubator. hypoxic pump 1 hr. after pump. put water bath  $37^\circ\text{C}$  1 hr. then radiation. after wash out the drug. put new medium. incubator 7 day later. stain

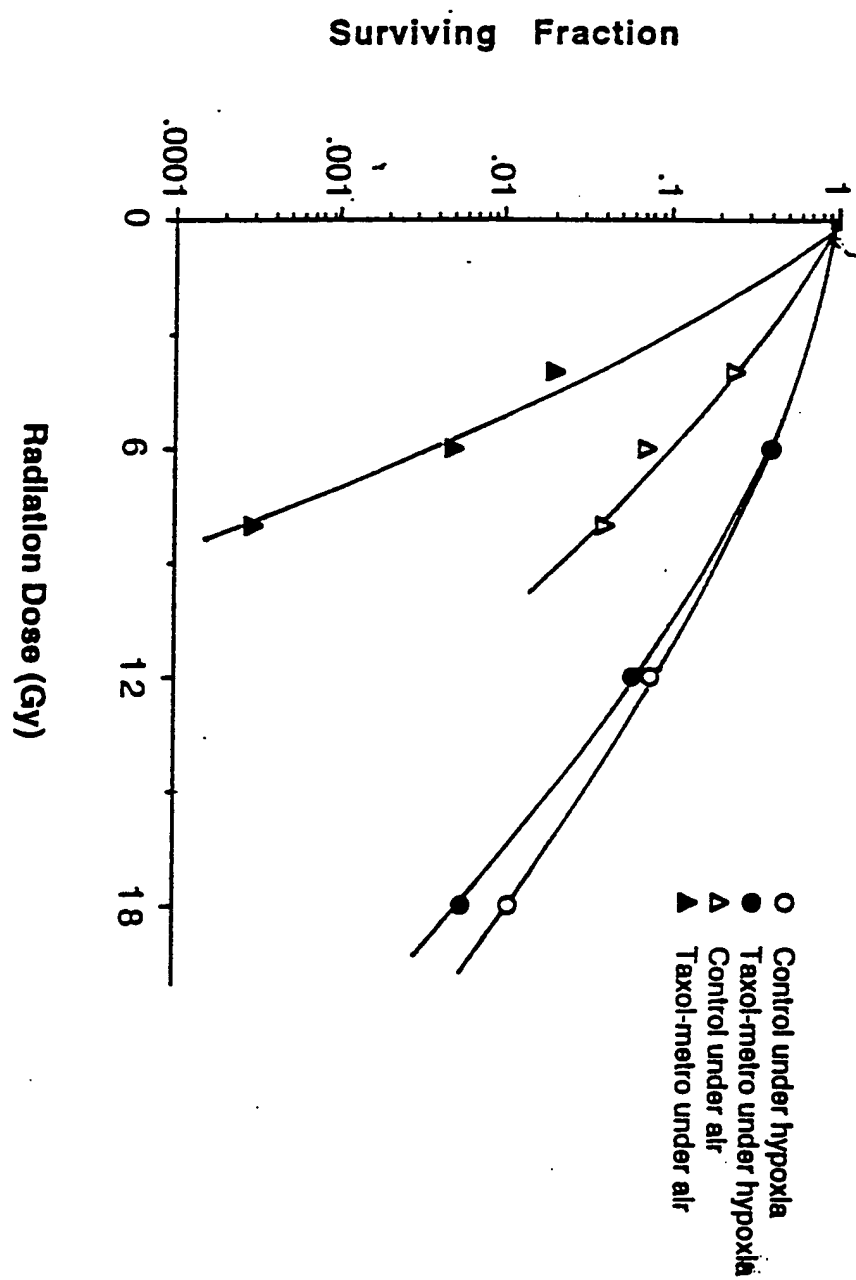
Drug from:  $10^{-3}$

$$20\mu\text{l} + 1.98\mu\text{l} = 10^{-5} \rightarrow 0.8\mu\text{l} + 7.2\mu\text{l} = 10^{-6} \rightarrow 8\mu\text{l} + 72\mu\text{l} = 10^{-7}$$

(14855)

$N = \text{Count}$	mean	SF	SF/PE	$N_2 + \text{Drug Count}$	mean	SF	SF/PE
SG 49.61.85	65	0.0065	0.011	189 101.79.22	67.3	0.003	0.0057
26 113.94.83	16.7	0.048	0.078	100.79.73	84	0.042	0.071
66 119.130.119	122.7	0.25	0.40	136.108.141	128.3	0.26	0.435
0 132.127.112	123.7	0.62 (PE)		125.128.103	118.7	0.59 (PE)	
$O_2 \text{ Count}$				$O_2 + \text{Drug}$			
86 51.56.63	56.7	0.028	0.04	0.1.0	0.3	0.0006	0.003
66 30.17.30	25.7	0.05	0.072	3.0.4.	2.3	0.001	0.005
46 38.25.44	35.7	0.18	0.25	8.3.3	4.7	0.0047	0.02
0 153.132.141	142	0.71 (PE)		48.36.47	43.7	0.22 (PE)	

Radiosensitizing Effects of Taxol-metro  
 (100nM, 2 hr treatment) on CHO Cells



1/2 E  
 7  
 1  
 5

3  
 5

## 5C PLDR EXPERIMENT

RT=86%

Drug=0.1mM

Cells

0 hr RT 22.000

2 hr RT 10.000

4 hr RT 5000

6 hr RT 2000

2 hr RT+Drug 10.000

4 hr RT+Drug 5000

6 hr RT+Drug 2000

2 hr ORT+Drug 200

4 hr ORT+Drug 200

6 hr ORT+Drug 200

Plated cell in dish. each dish put 200 cells. in incubator 3hr then add drug pump 1hr. after pump. put waterbath  $37^{\circ}\text{C}$  1hr. then wash off the drug. add new medium. incubator 8 days. then stain. count.

~~$$2\mu + 1/8\mu = 1.125$$~~

N <sub>2</sub> Count	mean	SF	SF/PE	O <sub>2</sub> Count	mean	SF	SF/PE	
SC <sub>1</sub>	0			0				
SC <sub>2</sub>	129.108.104	113.7	0.57	0.848	121.134.140	131.7	0.66	1.062
SC <sub>3</sub>	147.121.109	125.7	0.63	0.938	129.138.117	128	0.66	1.032
Q	142.129.131	134	0.67 (PE)		143.118.112	124.3	0.62 (PE)	
M <sub>1</sub>	122.134.129	128.3	0.64	0.958	136.130.122	129.3	0.65	1.043
M <sub>2</sub>	130.125.127	127.3	0.64	0.950	130.124.120	124.7	0.62	1.005
M <sub>3</sub>	133.130.119	127.3	0.64	0.950	120.126.109	118.3	0.59	0.954

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5C PLDR Experiment

5C = 0.1 mM

2W RT 2000

RT = 86f

Control 200

2W RT 5000

2W RT+D 5000 -

2W D 200

4W RT 5000

4W RT+D 5000

4W D 200

6W RT 2000

6W RT+D 2000

6W D 200

Nitro-phenyl-Tartrate (TNB) + Radiation			CHO cell			Drug = $10^{-7} M$
RT (Ct)	N <sub>2</sub> +D	N <sub>2</sub>	RT (Ct)	O <sub>2</sub> +D	O <sub>2</sub>	
18	20.000	10.000	8	10.000	5000	
12	2000	1000	6	5000	1000	
6	1000	500	4	2000	500	
0	200	200	0	200	200	

Plated cell in dish incubator 3hr then add drug pump 1hr. after post waterbath 37°C 1hr. then radiation optic in incubator.

Drug from TNB  $10^{-3}$  (1mm)  $\rightarrow 0.02 \text{ ml} + 1.98 \text{ ml} = 10^{-5}$

$\downarrow$   
1 ml + 9 ml =  $10^{-6}$

$\downarrow$   
8 ml + 72 ml =  $10^{-7}$

N <sub>2</sub> +D Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
1u 139. 95. 99	111	0.0056	0.0135	64. 78. 36	59.3	0.0059	0.0138
12u 105. 110. 110	108.3	0.054	0.13	84. 26. 52	54	0.054	0.13
60u 124. 104. 155	151	0.151	0.368	72. 71. 60	67.7	0.135	0.31
0 95. 97. 54	82	0.41 (PE)		110. 94. 55	86.3	0.43 (PE)	
O <sub>2</sub> +D Count				O <sub>2</sub> Count			
80u 74. 80. 73	52.3	0.005	0.009	42. 59. 61	54	0.0108	0.018
60u 148. 135. 130	137.7	0.028	0.0487	37. 54. 33	41.3	0.041	0.07
40u 171. 157. 182	170	0.085	0.15	64. 80. 78	74	0.148	0.25
0 112. 110. 117	113	0.565 (PE)		109. 120. 126	118.3	0.59 (PE)	

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5C PLDR Experiment

Drug = 0.1 mM  
RT = 86%

## Metro + Radiation

Drop = 0.5 mm.

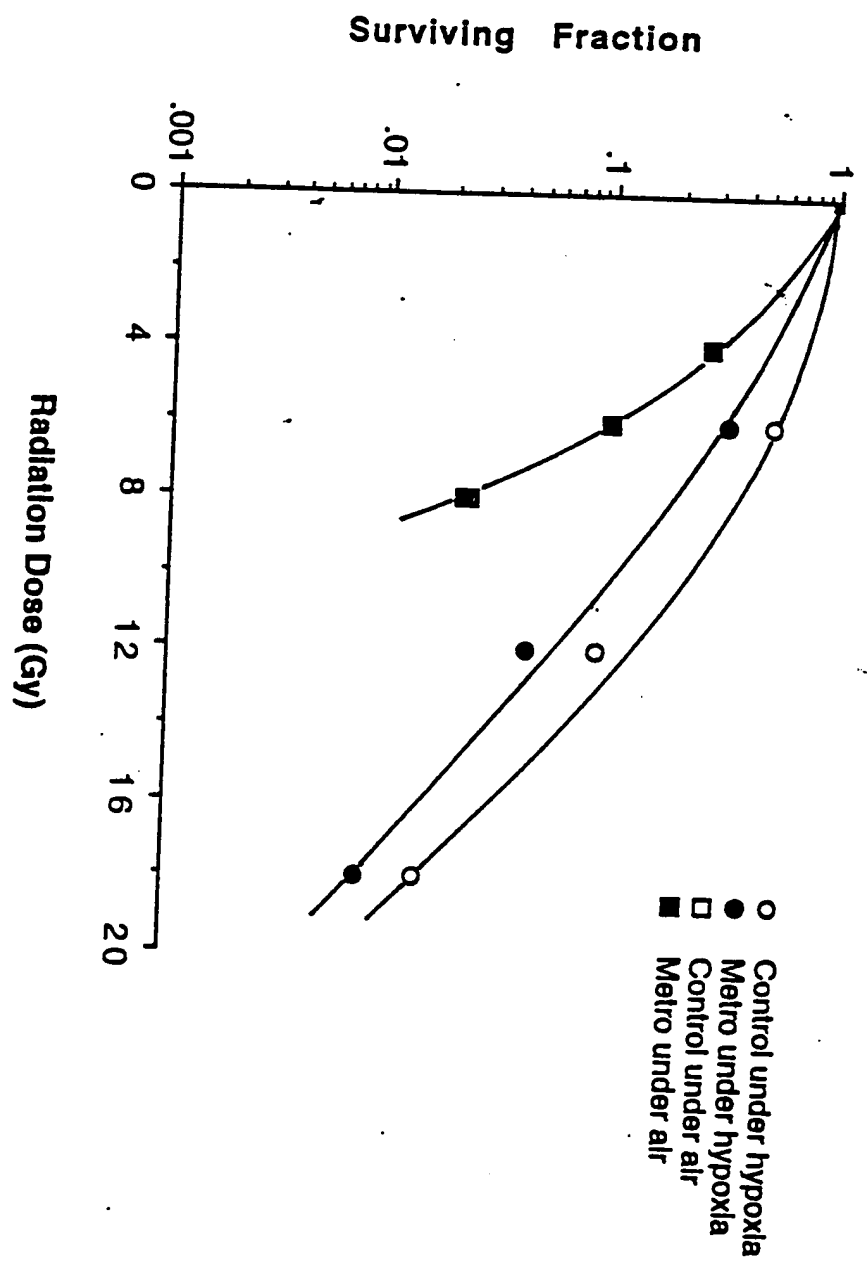
RT (G4)	N <sub>2</sub> +D	N <sub>2</sub>	RT (G4)	O <sub>2</sub> +D	O <sub>2</sub>
18	20.000	10.000	8	10.000	5000
12	2000	1000	6	2000	1000
6	500	200	4	1000	200
0	200	200	0	200	200

plated cells in dish incubator 3 hr then add drug pump 1 hr. then waterbath 37°C 1 hr.  
 then radiation.oxic in incubator. after radiation wash off the drug. add new medium incubate 8 hr.  
 Drug: marker. 6.84 mg/80 ml (HBSS) each dish 3 ml drug.

N <sub>2</sub> +D Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
18 G4 45.60.46	67	0.0034	0.008	64.83.52	66.3	0.0066	0.015
12 G4 46.28.41	38.3	0.019	0.045	43.66.14	41	0.041	0.093
6 G4 70.70.74	71.3	0.143	0.332	37.54.47	46	0.23	0.523
0 84.46.80	86.7	0.43 (PE)		80.83.101	88	0.44 (PE)	

O <sub>2</sub> +D Count	mean	SF	SF/PE	O <sub>2</sub> Count	mean	SF	SF/PE
8 G4 115.124.123	120.7	0.012	0.023	82.68.59	69.7	0.014	0.024
6 G4 108.108.104	106.7	0.053	0.1	57.63.55	58.3	0.058	0.102
4 G4 137.150.152	146.3	0.146	0.274	42.20.32	31.3	0.157	0.275
0 103.112.106	107	0.535 (PE)		118.106.119	114.3	0.57 (PE)	

Radiosensitizing Effects of Metronidazole (0.5 mM) on CHO Cells



## Metro + Radiation (2nd)

Drug = 0.5 mM.

RT (day)	N <sub>2</sub> + D	N <sub>2</sub>	RT (day)	O <sub>2</sub> + D	O <sub>2</sub>
18	20.000	10.000	8	10.000	5000
12	2000	1000	6	2000	1000
6	500	200	4	1000	200
0	200	200	2	200	200

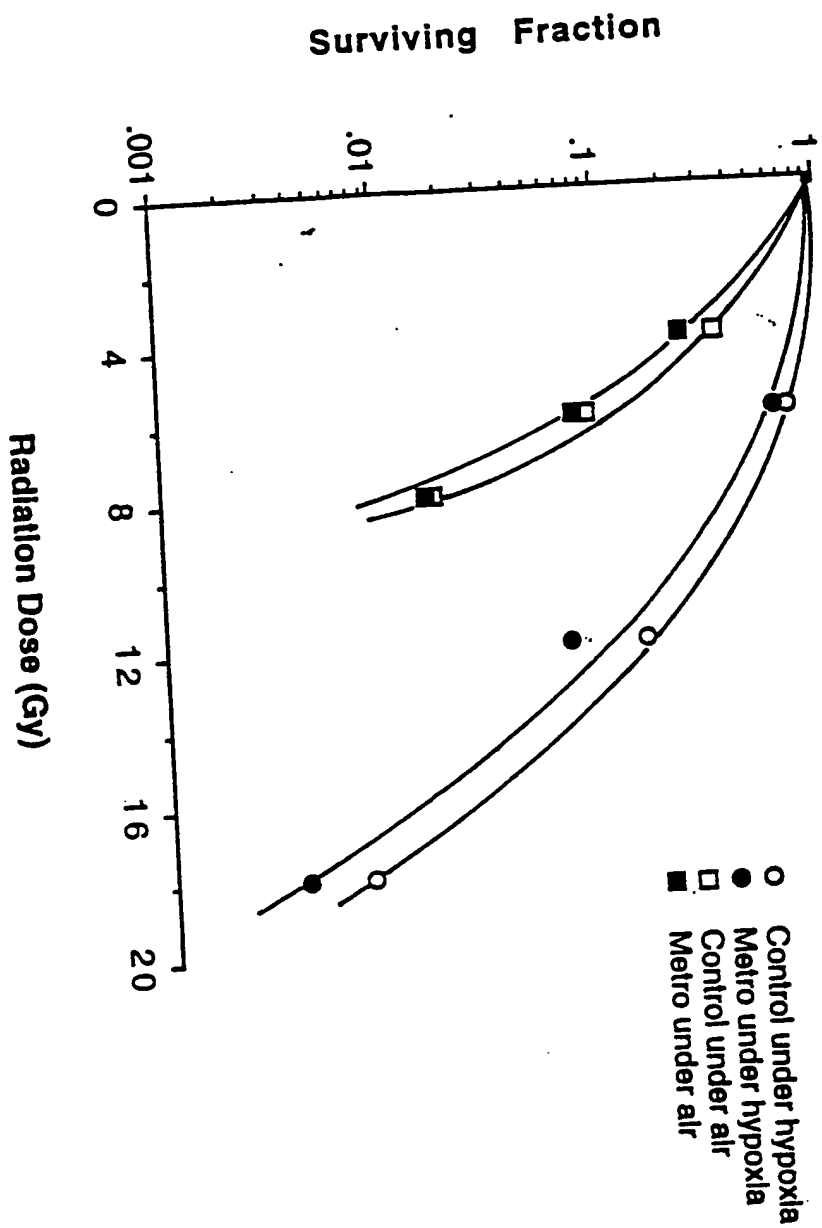
Plated Cell in dish incubator 3hr then add drug pump 1hr. after pump put waterbath 37°C  
1hr. then radiation. optic work in incubator. after radiation wash off the drug add new medium  
incubator 7 day stain.

Drug: Metro 6.8 ng/tond HBSS.

	N <sub>2</sub> + D Count	Mean	SF	SF/PE	N <sub>2</sub> Count	Mean	SF	SF/PE
186f	34.32.32	32.7	0.0016	0.004	37.46.26	36.3	0.0036	0.008
126f	49.50.65	54.7	0.027	0.069	66.67.60	64.3	0.064	0.147
66f	123.122.120	124.7	0.243	0.611	43.84.56	61	0.305	0.698
0	57.48.84	79.7	0.398 (PE)		70.79.113	87.3	0.437 (PE)	

	O <sub>2</sub> + D Count	Mean	SF	SF/PE	O <sub>2</sub> Count	Mean	SF	SF/PE
86f	132.134.102	122.7	0.012	0.016	62.64.64	63.3	0.013	0.017
66f	104.124.115	114.3	0.057	0.076	61.79.56	65.3	0.065	0.087
46f	187.204.149	180	0.18	0.24	52.42.59	51	0.255	0.339
0	141.138.171	150	0.75 (PE)		146.153.152	150.7	0.752 (PE)	

# **Radiosensitizing Effects of Metronidazole (0.5mM) on CHO Cells (2nd)**



114

37°C

24h

1

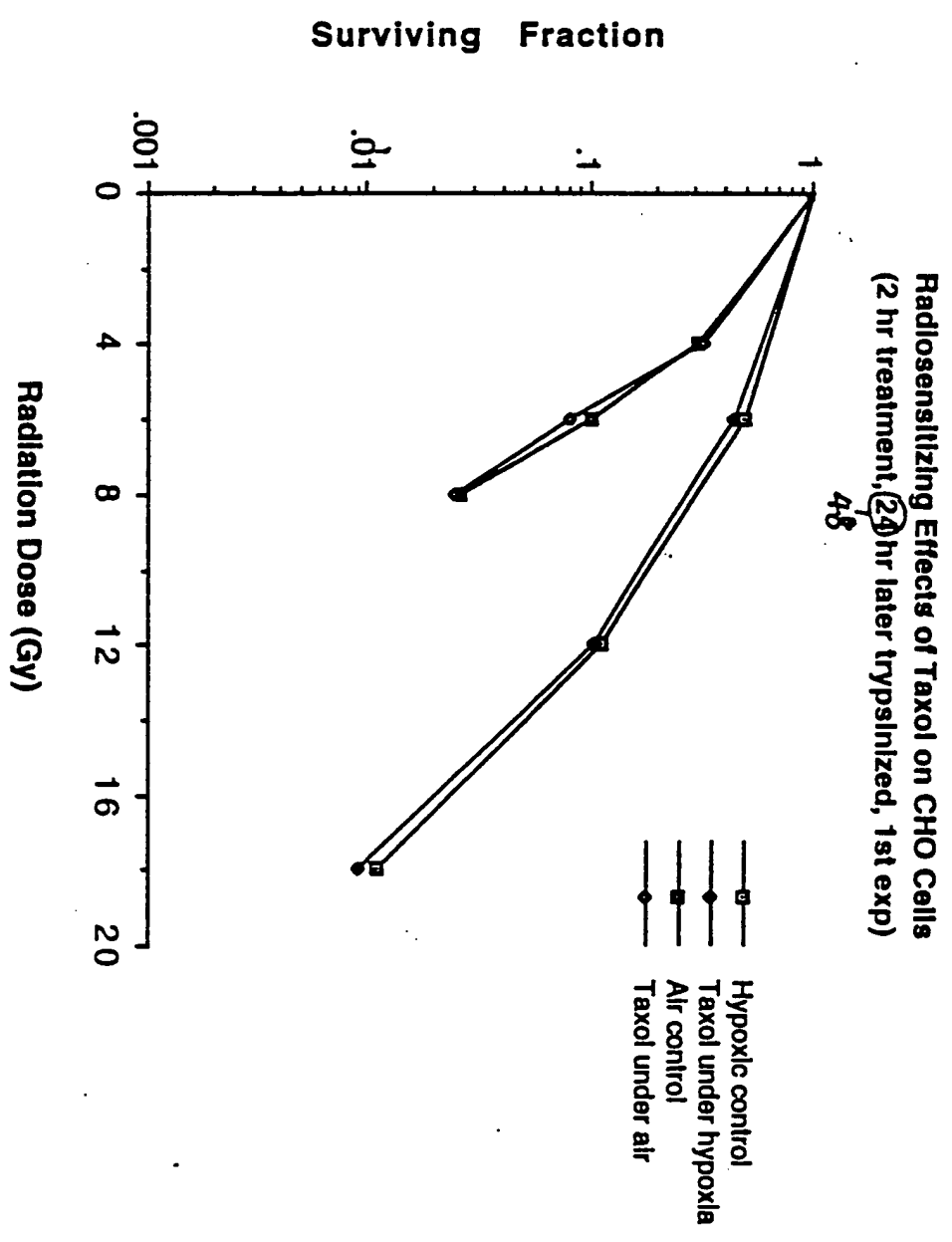
Target on CHO cells (2 hr treatment, 48 hr later trypsinized experiment)

plant cell in 2 dishes (each 22 mm)  $5 \times 10^4$ . one day later one dish add  $5 \times 10^6$  m.r. Taxol with HBSS. one dish add HBSS with DMSO 2 hr at  $37^\circ\text{C}$  incubator. then wash off the drug put new medium incubator 48 hrs. then trypsinize then plant cells in dish incubator. later 2 hours. then hypoxic 1 hr oric keep in incubator. then irradiate. after radiation put in incubator 7 days later stain. Count

RT (ct)	$N_2 + \text{Taxol}$	$N_2 + \text{HBSS}$	RT (ct)	$O_2 + \text{Taxol}$	$O_2 + \text{HBSS}$	Drug: from $10^{-3}$ (100 M)
18	10,000	10,000	8	2000	2000	$0.05 \mu\text{L} + 9.95 \mu\text{L} = 5 \times 10^{-6}$
12	2000	2000	6	1000	1000	Taxol (HBSS)
6	500	500	4	500	500	$0.05 \mu\text{L} + 9.95 \mu\text{L} = 10 \mu\text{L}$
0	200	200	0	200	200	DMSO HBSS

$N_2 + D$ Count	mean	SF	SF/PE	$N_2 + \text{HBSS}$ Count	mean	SF	SF/PE
186 61.45.71	59	0.0059	0.009	73.87.92	84	0.0084	0.011
126 124.159.115	132.7	0.066	0.104	165.186.172	174.3	0.087	0.113
66 150.174.130	138	0.276	0.435	174.191.193	186	0.372	0.482
0 137.120.124	127	0.635 (PE)		160.156.147	154.3	0.772 (PE)	

$O_2 + D$ Count	mean	SF	SF/PE	$O_2 + \text{HBSS}$ Count	mean	SF	SF/PE
86 30.35.20	28.3	0.014	0.035	40.44.41	41.7	0.021	0.026
66 62.36.39	45.7	0.046	0.08	89.74.75	79.3	0.079	0.1
46 83.92.98	91	0.182	0.318	116.117.124	119.	0.238	0.301
0 114.114.116	114.7	0.573 (PE)		160.161.153	158	0.79 (PE)	



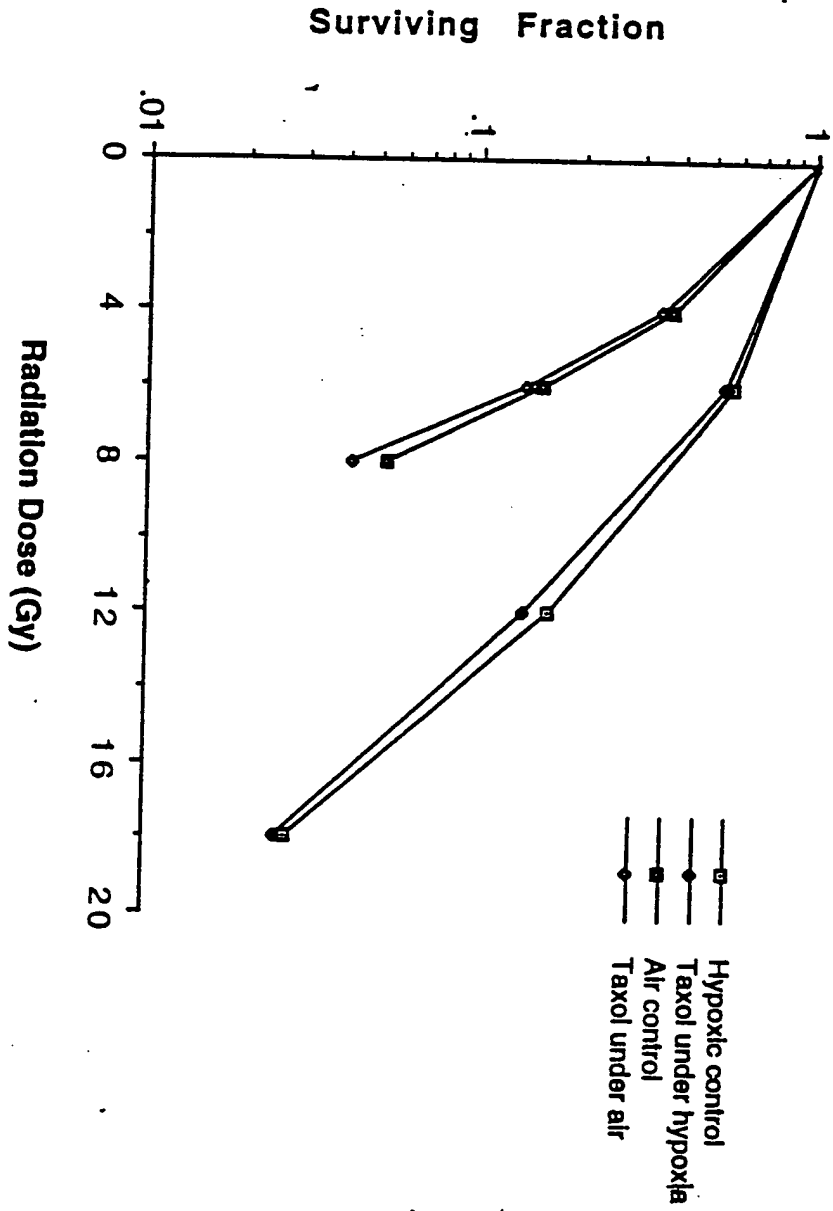
100%  
100%  
100%  
100%

Tabel on CHO cells (2 hr treatment 48 hr later trypsinized experiment)

Repeat Experiment

RT (hr)	N <sub>2</sub> +D	N <sub>2</sub> +HBSS	RT (hr)	O <sub>2</sub> +D	O <sub>2</sub> +HBSS	Length from total		
18	10.000	10.000	8	2000	2000	$10^{-3}$ (mm)		
12	2000	2000	6	1000	1000	$1.05 \mu\text{m} + 9.95 \mu\text{m} = 5.8 \times 10^{-6}$ (HBSS)		
6	500	500	4	500	500	$0.05 \mu\text{m} + 9.95 \mu\text{m}$ (HBSS)		
0	200	200	0	200	200			
N <sub>2</sub> +Total Count		mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
18 hr	112. 135. 87	108	0.0108	0.025	168. 186. 159	171	0.0171	0.027
12 hr	127. 119. 107	117.7	0.059	0.137	197. 209. 210	205.3	0.103	0.161
6 hr	124. 108. 112	114.7	0.229	0.533	187. 173. 183	181	0.362	0.568
0	77. 88. 94	86.3	0.43 (PE)		115. 122. 145	127.3	0.637 (PE)	
O <sub>2</sub> +D Count					O <sub>2</sub> Count			
8 hr	33. 34. 41	36	0.018	0.041	78. 82. 52	70.7	0.035	0.052
6 hr	59. 58. 65	60.7	0.061	0.138	106. 103. 99	102.7	0.103	0.152
4 hr	68. 75. 83	75.3	0.151	0.342	117. 132. 127	125.3	0.251	0.371
0	88. 86. 92	88.7	0.44 (PE)		126. 141. 138	135	0.675 (PE)	

**Radiosensitizing Effects of Taxol on CHO Cells  
(2 hr treatment, 48 hr later trypsinized, 2nd exp)**



1/  
1)  
 $\chi_{10}^{-6}$

	Total	T-B	on HCT cells	toxicity
	0.2	count	mean	
T-5	3 dishes	0.2		
T-6	"	0		
T-7	"	0		
T-8	"	2.0.0		
T-B-5	"	0.0.0		
T-B-6	"	0.0.0		
T-B-7	"	0.0.0		
T-B-8	"	1.0.0		
0	69.66.62	65.7	0.728 (PE)	

plant cell in dish incubator for 48 hrs. then add drug incubator 24 hrs. then wash off the drug. put new medium <sup>keep in incubator for</sup> 10 days stain.

Drug Total from  $10^{-3}$  (1 mm)

$$\begin{array}{lcl}
 0.18 \mu\text{l} + 2.39 \mu\text{l} = 7 \times 10^{-5} & \text{each dish add} & 0.5 \mu\text{l} + 3 \mu\text{l} = 10^{-5} \\
 \downarrow & & \text{(old)} \\
 0.2 \mu\text{l} + 1.8 \mu\text{l} = 7 \times 10^{-6} & \text{each dish add} & 0.5 \mu\text{l} + 3 \mu\text{l} = 10^{-6} \\
 \downarrow \text{(HBSS)} & & \text{medium} \\
 0.2 \mu\text{l} + 1.8 \mu\text{l} = 7 \times 10^{-7} & \text{each dish add} & 0.5 \mu\text{l} + 3 \mu\text{l} = 10^{-7} \\
 \downarrow & & \\
 0.2 \mu\text{l} + 1.8 \mu\text{l} = 7 \times 10^{-8} & \text{each dish add} & 0.5 \mu\text{l} + 3 \mu\text{l} = 10^{-8}
 \end{array}$$

T-B from  $10^{-3}$  (1 mm)

$$\begin{array}{lcl}
 0.18 + 2.39 \mu\text{l} = 7 \times 10^{-5} & \text{each dish add} & 0.5 \mu\text{l} + 3 \mu\text{l} = 10^{-5} \\
 \downarrow & & \\
 0.2 \mu\text{l} + 1.8 \mu\text{l} = 7 \times 10^{-6} & \text{each dish add} & 0.5 \mu\text{l} + 3 \mu\text{l} = 10^{-6}
 \end{array}$$

## Nitro-phenyl-tetrazol + Radiation on CHO cells

TVB =  $10^7$ 

RT	N <sub>2</sub> +D	N <sub>2</sub>	RT	O <sub>2</sub> +D	O <sub>2</sub>
18	201000	10.000	8	5000	2000
12	5000	2000	6	2000	1000
6	1000	500	4	1000	500
0	200	200	0	200	200

plant cell in dish incubator for 6 hrs. then add drug pump 1 hr. after pump with chamber put incubator <sup>for</sup> 24 hrs then irradiate after radiation wash off the drug put new medium. incubator 7 days stain

$$\text{Drug} = \text{TVB } 1 \text{ mM} \rightarrow 0.02 \text{ mL} + 1.98 \text{ mL} = 10^{-5}$$

$$\downarrow$$

$$1 \text{ mL} + 9 \text{ mL} = 10^{-6}$$

$$\downarrow$$

$$10 \text{ mL} + 90 \text{ mL} = 10^{-7}$$

N <sub>2</sub> +D Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
186 88.89.83	86.7	0.004	0.0088	81.78.70	76.3	0.0076	0.0141
126 206.177.207	196.7	0.039	0.08	147.102.172	153.7	0.077	0.142
66 243.205.199	229	0.229	0.467	205.190.172	189	0.378	0.7
0 109.100.85	98	0.49 (PE)		102.123.101	108.7	0.54 (PE)	
O <sub>2</sub> +D Count				O <sub>2</sub> Count			
86 51.50.74	58.3	0.012	0.046	125.123.123	123.7	0.062	0.097
66 60.70.73	62.7	0.0338	0.133	196.210.207	204.3	0.204	0.321
46 70.74.79	74.3	0.074	0.292	183.192.191	188.7	0.377	0.592
0 53.47.53	51	0.255 (PE)		132.118.132	127.3	0.637 (PE)	

# Taxol and Taxol-B Toxicity on HCT cells.

T=Taxol	Plant cell	Count	Taxol-B.	Plant cells	Count
$T-10^{-6}$	500	0	$T-B-10^{-6}$	500	0
$T-10^{-7}$	500	1.0.0	$T-B-10^{-7}$	500	1.0.0
$T-10^{-8}$	500	2.3.1	$T-B-10^{-8}$	500	0.0.1
$T-10^{-9}$	500	0.0.0	$T-B-10^{-9}$	500	1.1.1
$T-10^{-10}$	500	5.0.2	$T-B-10^{-10}$	500	1.2.1

Control: 200 150.150.152

plant HCT cells in dish, each dish 500 cells. Control 200 cells. incubator 5.5 hr later add 0.5ml drug. total 3.5ml each dish. drug in medium. then keep incubator 24 hrs. with drug later wash off the drug. add new medium keep incubator for 9 days. stain.

Drug: from: Taxol. Taxol-B.  $10^{-3}$  (mm)

$$0.12 \mu\text{L} + 1.59 \mu\text{L} = 7 \times 10^{-5}$$

$$0.18 \mu\text{L} + 2.39 \mu\text{L} = 7 \times 10^{-5}$$

$$0.2 \mu\text{L} + 1.8 \mu\text{L} = 7 \times 10^{-6}$$

$$0.2 \mu\text{L} + 1.8 \mu\text{L} = 7 \times 10^{-7}$$

$$0.2 \mu\text{L} + 1.8 \mu\text{L} = 7 \times 10^{-8}$$

$$0.2 \mu\text{L} + 1.8 \mu\text{L} = 7 \times 10^{-9}$$

$$0.2 \mu\text{L} + 1.8 \mu\text{L} = 7 \times 10^{-10}$$

each dish add 0.5ml with dish medium 3ml. total 3.5ml  
(drug)  $= 10^{-6}$

## T-M and TNB in HeT cell toxicity

T-M	cells	count	mean	SF	SF/PE
T-M $10^{-7}$	500	0	0		
T-M $10^{-8}$	500	10.4.6	6.7	0.073	0.019
T-M $10^{-9}$	500	212.235.197	214.7	0.429	0.609
T-M $10^{-10}$	500	221.222.243	228.7	0.457	0.649
TNB $10^{-7}$	500	0 0 0			
TNB $10^{-8}$	500	7.5.6	6	0.012	0.07
TNB $10^{-9}$	500	174.215.160	183	0.366	0.519
TNB $10^{-10}$	500	187.189.178	184.7	0.369	0.524
?	200	121.143.159	141	0.705 (PE)	

plant 500 HeT cells each dish in incubator for 6hr. then add drug with medium keep in incubator 24hrs. later wash off the drug. add new medium incubator keep for 9 days stim.

Drug from  $10^{-3}$  (1mm)

$$11 \mu\text{l} + 9.98 \mu\text{l} = 1.1 \times 10^{-6}$$

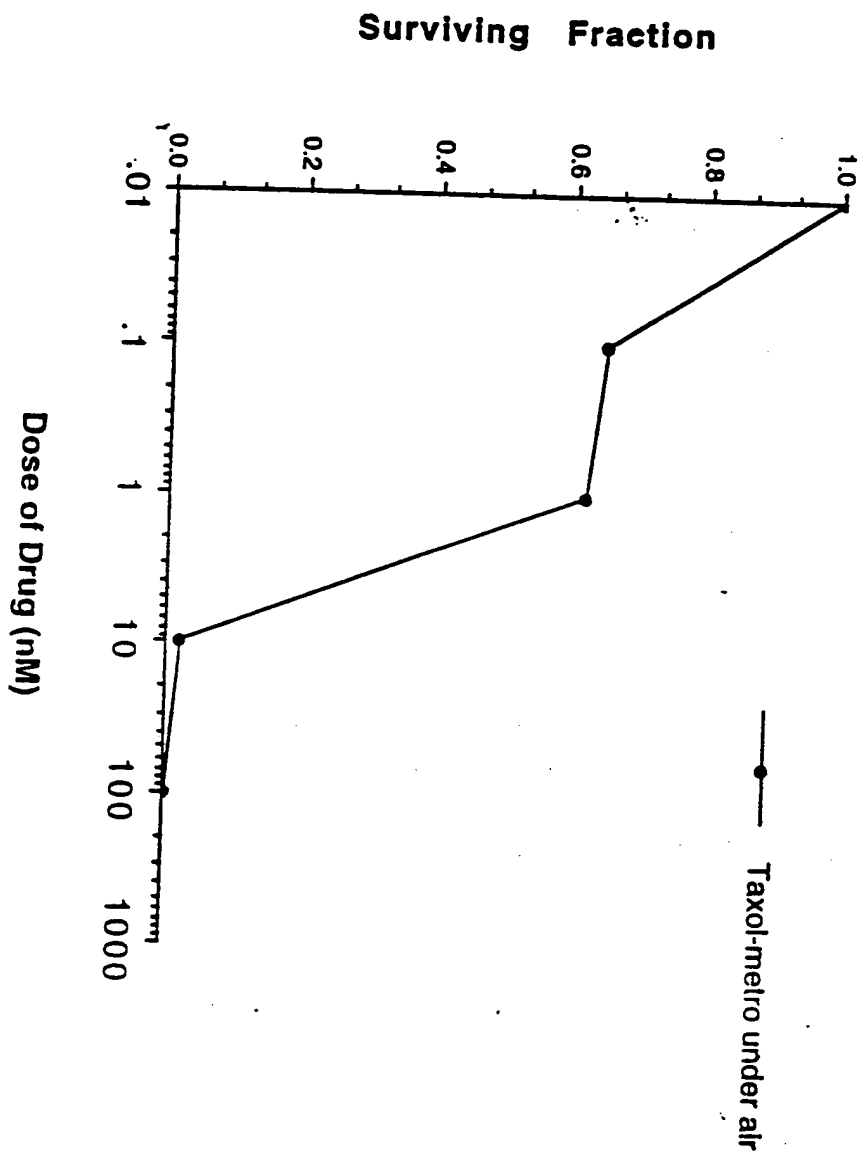
$$0.2 \mu\text{l} + 1.8 \mu\text{l} = 1.1 \times 10^{-7}$$

$$0.2 \mu\text{l} + 1.8 \mu\text{l} = 1.1 \times 10^{-8}$$

$$0.2 \mu\text{l} + 1.8 \mu\text{l} = 1.1 \times 10^{-9}$$

→ each dish add  $0.3 \mu\text{l} + 3 \mu\text{l} = 10^{-7}$   
(drug) (old medium)

Ch moth rap ulic Activities of  
Taxol-metro against HCT Cancer Cells



Taxol-Metvix + Radiation (Repeat Experiment)  $D=10^{-7}$

RT	N <sub>2</sub> +D	N <sub>2</sub>	D <sub>2</sub> +D	O <sub>2</sub>
180f	20.000	10.000	80f 5000	2000
120f	2000	2000	60f 2000	500
60f	500	500	40f 1000	200
0	200	200	0 200	200

Plant CHO cells in dialy incubator for 3hr. then add drug with HBSS pump 1hr. edic in incubator. after pump, put waterbath 37°C 1hr. then irradiate. after RT. wash off the drug. add new medium keep incubator 2 days. stain.

Drug: from T-M. Taxol  $10^{-3}$  (100mM)

$$\downarrow$$

$$200\mu\text{L} + 1.98\mu\text{L} = 10^{-5}$$

$$\downarrow$$

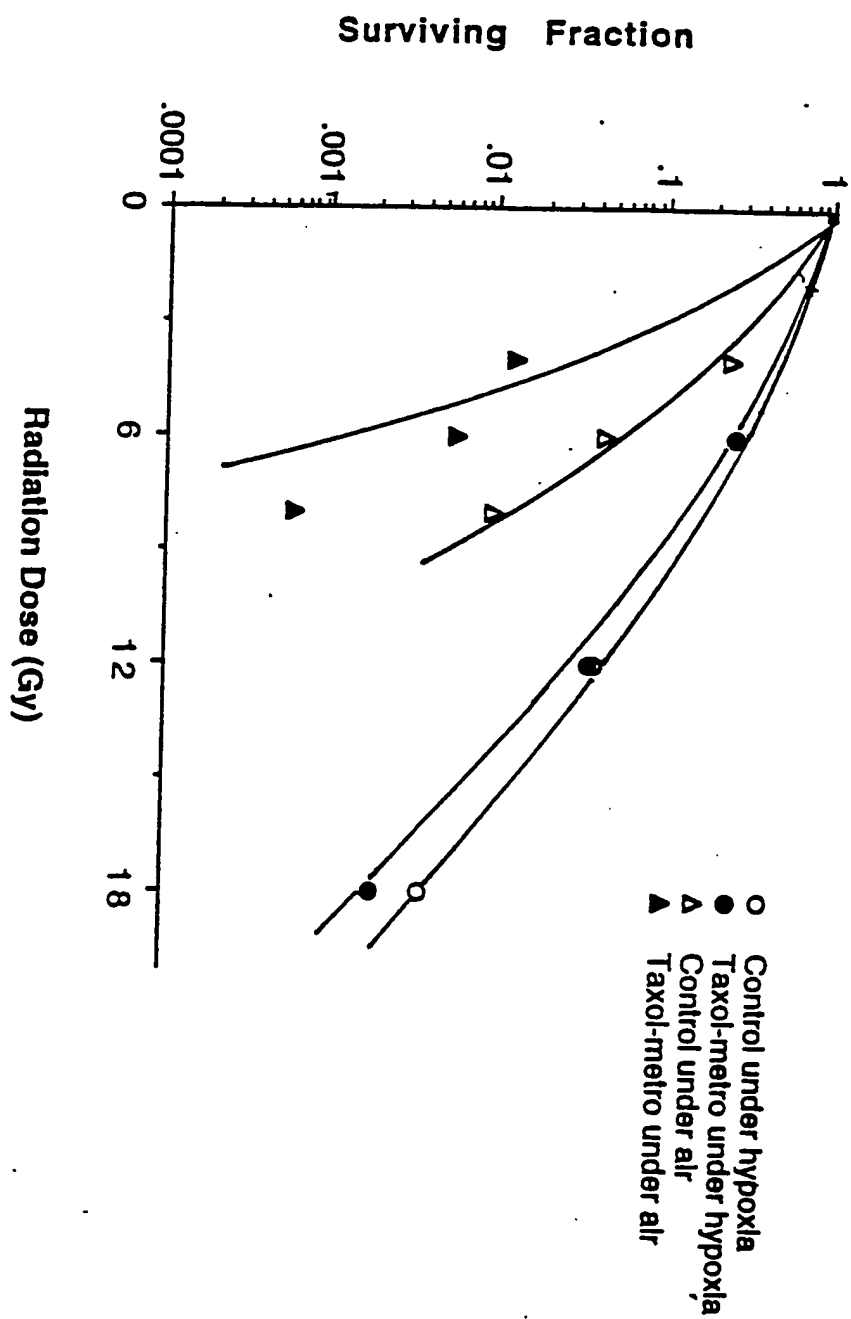
$$0.8\mu\text{L} + 7.2\mu\text{L} = 10^{-6}$$

$$\downarrow$$

$$8\mu\text{L} + 72\mu\text{L} = 10^{-7}$$

	N <sub>2</sub> +D Count	mean	sf	sf/pe	N <sub>2</sub> Count	mean	sf	sf/pe
180f	27. 20. 21	22.7	0.001	0.002	27. 40. 34	24.7	0.0025	0.004
120f	41. 44. 48	43.3	0.022	0.037	57. 58. 44	53	0.0265	0.041
60f	84. 88. 62	78	0.156	0.27	104. 74. 77	85	0.17	0.264
0	111. 120. 116	115.7	0.578 (PE)		120. 126. 140	128.7	0.643 (PE)	
	O <sub>2</sub> +D Count				O <sub>2</sub> Count			
80f	1. 0. 0	0.3	0.00007	0.0006	12. 9. 12	11	0.0055	0.01
60f	1. 1. 2	1.3	0.0007	0.006	13. 12. 13	12.7	0.025	0.045
40f	1. 1. 2	1.3	0.001	0.013	29. 23. 30	27.3	0.137	0.244
0	18. 23. 21	20.7	0.103 (PE)		118. 104. 115	112.3	0.56 (PE)	

**Radiosensitizing Effects of Taxol-metro  
(100 nM, 2 hr treatment) on CHO Cells**



hr.  
64h

total-Metro + Radiation on CHO cells (Repeat Experiment)

RTG	N <sub>2</sub> +D	N <sub>2</sub>	RTG	O <sub>2</sub> +D	O <sub>2</sub>
18	20.000	10.000	8	5000	2000
16	5000	2000	6	2000	1000
12	1000	500	4	1000	500
0	200	200	0	200	200

plant cell in dish incubator for 6 hrs. then add drug with HBSS pump 1 hr. or in incubator after pump. put chamber into incubator for 24 hrs. then take out radiation. after RT. wash off the drug. add new medium to keep in incubator for 8 days. then stain it.

Drug: from T-M. TNB  $10^{-3}$  (1 mm)

$$2.0 \mu\text{L} + 1.98 \mu\text{L} = 10^{-5}$$

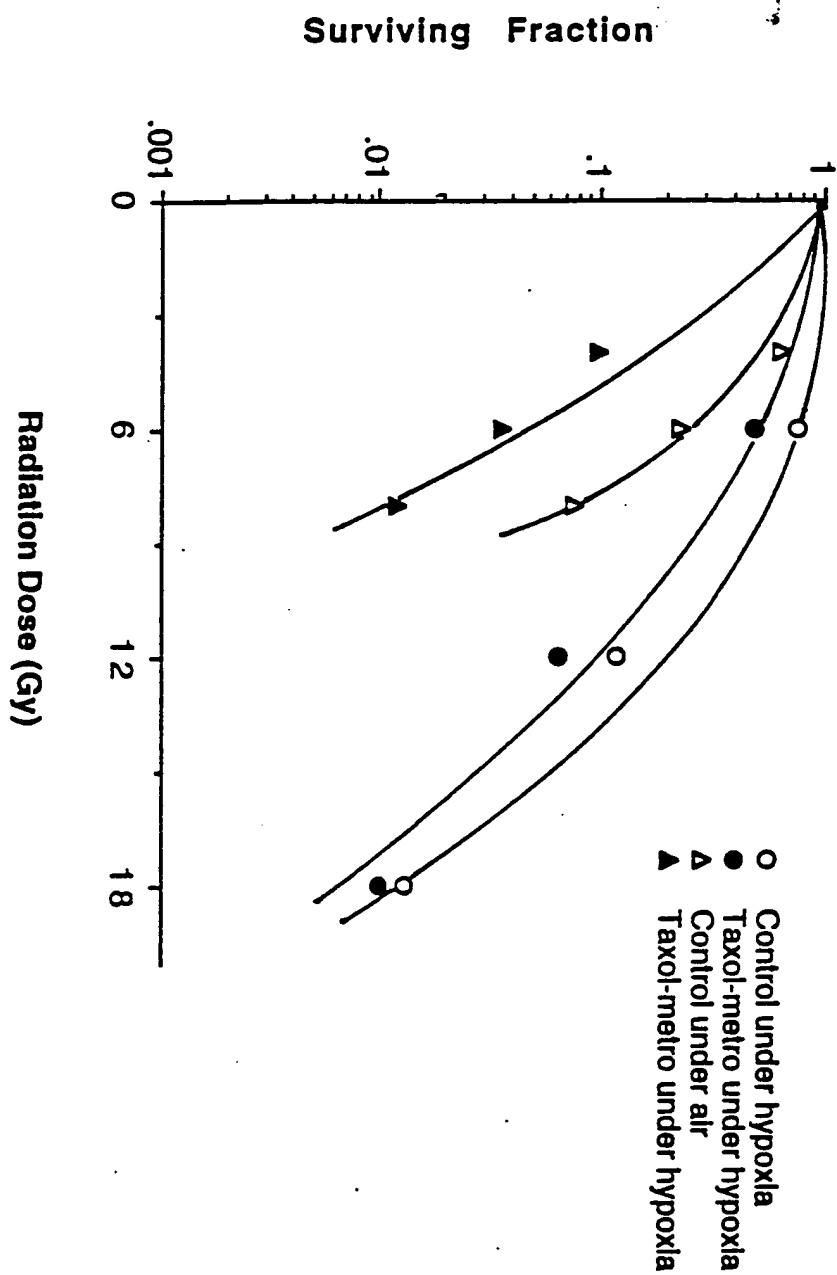
$$0.8 \mu\text{L} + 7.2 \mu\text{L} = 10^{-6}$$

$$0.2 \mu\text{L} + 7.2 \mu\text{L} = 10^{-7}$$

each dish add 3  $\mu\text{L}$  drug with HBSS.

	N <sub>2</sub> +D Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
18Gy	35.45.55	45	0.00275	0.0099	56.48.31	45	0.0045	0.013
12Gy	57.71.91	73	0.0146	0.064	86.109.58	84.3	0.042	0.118
6Gy	81.113.137	110.3	0.11	0.484	162.128.113	134.3	0.269	0.753
0	35.65.24	45.7	0.228 (PE)		62.80.72	71.3	0.357 (PE)	
	O <sub>2</sub> +D Count	mean	SF	SF/PE	O <sub>2</sub> Count	mean	SF	SF/PE
8Gy	2.5.3	3.3	0.0007	0.012	101.77.78	83.3	0.043	0.077
6Gy	6.3.3	4	0.002	0.036	123.125.137	128.3	0.128	0.25
4Gy	3.4.9	5.3	0.005	0.097	156.190.187	177.7	0.355	0.638
0	16.6.11	11	0.055 (PE)		124.110.100	111.3	0.557 (PE)	

Radiosensitizing Effects of Taxol-metro  
(100nM, 24hr treated) on CHO Cells (2nd EXP)



Total + RT on HCT cell

Drug =  $10^{-9}$ 

RT	N <sub>2</sub> + D	N <sub>2</sub>	RT	O <sub>2</sub> + D	O <sub>2</sub>
1864	20.000	20.000	864	20.000	20.000
1264	10.000	10.000	664	10.000	10.000
664	5000	5000	464	5000	5000
0	200	200	0	200	200

Plant HCT cell in dish incubator for 4 hrs. then add drug hypoxic pump 1 hr. after pump. with chamber put incubator 24 hrs. etc add drug keep in incubator 24 hrs. later x-ray. after x-ray wash off the drug. add new medium keep in incubator 9 days. then stain it and count

Drug total: from  $10^{-7}$  (1 mm)  $\rightarrow$  11 ul + 9.98 ul (HBSS) =  $1.1 \times 10^{-6}$

$$0.2 \text{ ul} + 1.8 \text{ ul} = 1.1 \times 10^{-7}$$

$$0.8 \text{ ul} + 7.2 \text{ ul} = 1.1 \times 10^{-8} \text{ each dish add } 0.3 \text{ ul} + 3 \text{ ul} = 10^{-9} \text{ (with medium)}$$

RT	N <sub>2</sub> + D Count	mean	SF	SF/PE	N <sub>2</sub> Count	mean	SF	SF/PE
1864	0	0	0	0	0	0	0	0
1264	3.2.2.	2.3	0.0002	0.00076	7.5.7	7.3	0.0007	0.00197
664	42.35.28	35	0.007	0.0228	45.43.58	48.7	0.0097	0.0262
0	70.53.61	61.3	0.307 (PE)		74.80.69	74.3	0.372 (PE)	
O <sub>2</sub> + D Count								
364	9.14.14	12.3	0.0006	0.0013	14.15.20	16.3	0.00082	0.0015
664	25.60.54	63	0.0063	0.0132	120.97.100	105.7	0.0106	0.019
1264	200.187.198	195	0.039	0.082	278.281.249	269.3	0.054	0.097
0	45.79.112	45.3	0.477 (PE)		17.107.108	110.7	0.553 (PE)	

Taxol, Taxol-t-bu, Taxol-Metro, Nitrophenyl-taxol on HCT cells under air toxicity

Taxol	Count	mean	9F	SF/PE
T $2 \times 10^{-8}$	1.0.0	0.5	0.0025	0.0046
T $4 \times 10^{-9}$	4.1.1	2	0.001	0.0185
T $8 \times 10^{-10}$	81.74.76	77	0.385	0.71
T $1.6 \times 10^{-10}$	107.11.118	112	0.56	1.0332

Taxol-t-bu

T-B $2 \times 10^{-8}$	1.3	2	0.01	0.0185
T-B $4 \times 10^{-9}$	0.1.0	0.33	0.007	0.0031
T-B $8 \times 10^{-10}$	2.2.1	1.667	0.0083	0.0154
T-B $1.6 \times 10^{-10}$	43.35.35	37.7	0.1883	0.3425

Taxol-Metro

T-M $2 \times 10^{-8}$	0.0.0	0		
T-M $4 \times 10^{-9}$	1.2.1	1.33	0.007	0.0123
T-M $8 \times 10^{-10}$	2.4.4	3.3	0.07	0.031
T-M $1.6 \times 10^{-10}$	0.2.1	1	0.005	0.0092
TNB $2 \times 10^{-8}$	0.0.0	0	0	0
TNB $4 \times 10^{-9}$	0.0.1	0.3	0.0017	0.0031
TNB $8 \times 10^{-10}$	0.1.1	0.667	0.003	0.0062
TNB $1.6 \times 10^{-10}$	2.1.1	1.33	0.007	0.0123

Control 100.11.114 108.3 0.542 (PE)

Plant HCT cells in dish incubator 6hr. then add drug with medium keep in incubator for 24hrs. then wash off the drug. add new medium incubator for 9 days. stain.

drug. T. T-B. T-M. TNB from  $10^{-3}$  (1ml)

$$25 \mu\text{l} + 2.475 = 10^{-5}$$

$$0.5 \mu\text{l} + 4.5 \mu\text{l} = 10^{-6}$$

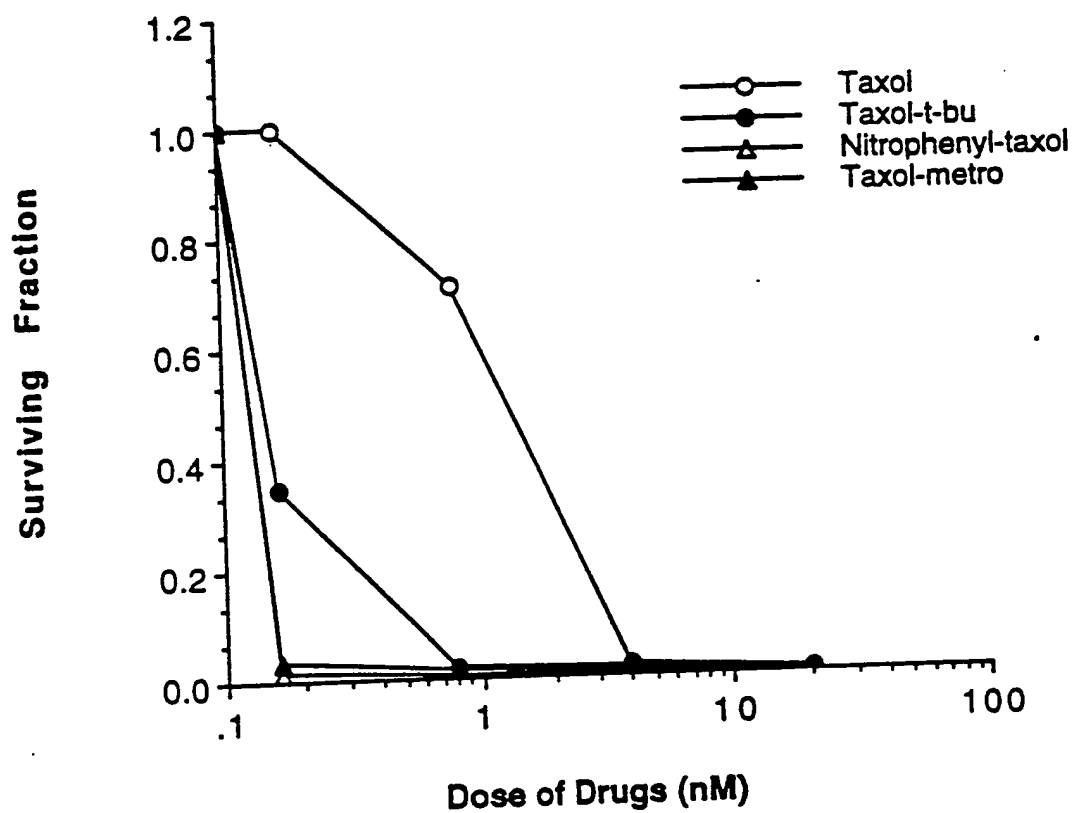
$$0.5 \mu\text{l} + 4.5 \mu\text{l} = 10^{-7}$$

$$3 \mu\text{l} + 12 \mu\text{l} = 4 \times 10^{-9}$$

$$3 \mu\text{l} + 12 \mu\text{l} = 8 \times 10^{-8}$$

$$2 \mu\text{l} + 12 \mu\text{l} = 1.6 \times 10^{-10}$$

Chemotherapeutic activities of Taxol, Taxol-t-bu,  
Nitrophenyl-taxol, and Taxol-metro on HCT116 Cells  
under air condition (1st exp)



T-B on HCT cell + Radiation -

T-B =  $5 \times 10^8$ 

RTG	$N_2 + D$	$N_2$	RTG	$N_2 + D$	$O_2$
18	40000	40000	8	20.000	40000
12	20.000	20.000	6	10.000	10.000
6	5000	5000	4	2000	2000
0	200	200	0	200	200

plant HCT cells in dish incubated for 6 hrs. then add drug pump 1 hr. sit in incubator. after pump, put incubator 23 hrs. total with drug for 24 hrs. then radiation. later wash off the drug. add new medium incubator for 9 days. then stain. count.

Drug from T-B ( $10^{-3}$  (1mm))

$$11 \mu\text{L} + 9.98 \mu\text{L} (\text{HBS}) = 1.1 \times 10^{-6}$$

$$0.4 + 3.6 \mu\text{L} = 1.1 \times 10^{-7}$$

$$1 \mu\text{L} + 1 \mu\text{L} = 5.5 \times 10^{-8}$$

each dish add  $0.3 \mu\text{L} + 3 \mu\text{L} = 5 \times 10^{-9}$   
(drug) (old medium)

	$N_2 + D$ Count	Mean	SF	SF/PE	$N_2$ Count	Mean	SF	SF/PE
18G	0.1.1	0.67	0.00017	0.009	6.3.2	3.7	0.000917	0.0002
12G	1.3.0	1.3	0.0007	0.00074	14.8.8	10	0.0005	0.00098
6G	6.19.11	12	0.0024	0.027	30.53.33	38.7	0.0077	0.015
0	9.26.17	17.3	0.09 (PE)		102.81.123	102	0.51 (PE)	

 $O_2 + D$  Count $O_2$  Count

18G	0	21. 17. 15	17.7	0.0004	0.0007
12G	0	107. 114. 118	113	0.0113	0.018
6G	0	159. 142. 189	180	0.09	0.163
0	0	114. 157. 128	126.3	0.63 (PE)	

No fig.

1094 302

TITLE:

DRUG:

Investigator:

P:2

Date:

Tumor size in mm

Group	drug dose	radiation dose	#	92	13	14	15	16	17	18	19	20	21	22	23
A TM Control			1	10.5	11.5	12.0	13.0	14.0	14.5	14.5	dead	X	X	X	X
			2	7.5	7.5	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.0	9.0	9.5
			3	9.5	10.0	10.5	11.5	12	13	14	14	14	15.0	16.0	16.0
B TM + 226g RT			4	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
			5	7.5	7.5	7.5	8.0	8.0	8.0	9.0	9.0	9.0	9.5	10.0	10.0
			6	7.5	7.5	7.5	7.5	8.0	8.0	8.0	8.0	8.0	9.0	10.0	10.0
C 226g RT Control			1	16	16	17.0	17.0	17.0	17.0	18.0	18.0	18.0	18.0	18.0	18.0
			2	12.5	13	13.0	14.0	14.0	14.0	15.0	16.0	16.0	17.0	17.0	17.0
			3	11	11	12.0	12.5	13.0	13.0	13.0	13.0	13.0	13.0	13.0	14.0
			4	13	14	14.0	14.0	14.0	14.0	14.0	15.0	16.0	16.0	16.0	16.0
			5	15	dead	X									
			6	15	16	dead									
D TM Control			1	7.5	7.5	8.0	8.0	8.0	8.5	9.5	10.0	11.0	12.0	12.0	12.0
			2												
			3												
			4	9.0	9.0	10.5	12.0	13.0	13.0	14.0	16.0	17.0	17.0	17.0	17.0
			5	7.0	7.0	7.0	7.0	8.0	8.0	8.0	8.0	8.0	8.0	8.5	8.5
			6	7.0	7.0	7.0	7.5	8.0	9.0	9.0	9.0	10.0	11.0	11.0	11.5
E TM + 226g RT			1												
			2	15	dead										
			3												
			4												
			5												
			6												
F Taxol + 226g RT			1												
			2	15	dead										
			3												
			4												
			5												
			6												
G Taxol Control			1												
			2												
			3												
			4												
			5												
			6												
H Taxol Control			1												
			2												
			3												
			4												
			5												
			6												
I vehicle Control			1	14	14	14	15	15	16	17	18	18			
			2	15.5	16	16.0									
			3	14	14	15	15	dead							
			4	16.5	17	18	18	18							
			5	15.5	16	16	17	17	17	18	18				
			6	16	16	16	dead								

711, LE:

DRUG :

Investigator :

$p:3$

Date:

Tumor size in mm

[illegible]

415 750 6215 P.07

[illegible]

17164

DRUG :

TM drug

Investigator:

P: 1

Date: [REDACTED] Tumor size in mm

[illegible]

UG : TM drug  
Investigator: P:2

Date: [REDACTED] Tumor size in mm

[illegible]

415 750 6215 P.08

investigator: p:3

Date: [REDACTED] Tumor size in mm

[illegible]

[illegible]

Time  
size

**MOUSE2.XLS**

Pick  
TVB

**MOUSE2.XLS**

[illegible]

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DRUG :

Date:

[illegible]

Investigator: TNB Control Date: [REDACTED]

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